UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Medicine

The primary prevention of asthma and associated allergic disease

by

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Thesis for the degree of Doctor of medicine

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ABSTRACT

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THE PRIMARY PREVENTION OF ASTHMA AND ASSOCIATED ALLERGIC DISEASE

Martha Scott

Asthma is a chronic disease that often starts in early childhood. The key risk factors are a child's environment and their genetic characteristics. We hypothesised that modification of environmental factors in infancy would continue to reduce the prevalence of asthma in a group of high-risk individuals in early adult life.

In 1990, 120 children considered at high risk of asthma due to either dual heredity or single heredity and a high cord total IgE, were enrolled in a single-blinded, randomised control trial. Those in the prevention arm (n =58) underwent dietary modification and house dust mite avoidance for the first 9 months of life. Follow-up occurred at age 1, 2, 4, 8 and 18 years.

At the 18-year follow-up assessment, there was a significant reduction in asthma in the prevention group compared the control group, 6 (10.7%) versus 15 (25.9%) ($p < 0.05$); this was explained by differences in atopic, rather than non-atopic, asthma. There were no differences in the rate of atopy at 18 years, unlike at previous assessments. Longitudinal analysis demonstrated significantly reduced prevalence of both asthma and atopy in the prevention group over the length of the follow-up.

Primary prevention of asthma by a combination of dietary and environmental modification successfully reduces the prevalence of asthma in high-risk individuals, and this effect is sustained into early adulthood. This may be due to a delayed onset of atopy thus preventing atopy from acting as a driver for asthma pathogenesis in childhood.

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ORIGINAL ARTICLE

Multifaceted allergen avoidance during infancy reduces asthma during childhood with the effect persisting until age 18 years

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ABSTRACT

Background Asthma is a chronic disease that often starts in childhood. The key risk factors are a child's environment and their genetic characteristics. The aim of this study was to evaluate the efficacy of environmental modification in the first 12 months of life on the prevalence of asthma in high-risk individuals.

Methods Children (n=120) considered at high risk of allergic disorders (either dual heredity or single heredity and a high cord total IgE), were enrolled in a single-blinded, randomised controlled trial. Infants in the intervention arm were either breast fed with the mother on a low allergen diet or given an extensively hydrolysed formula. Exposure to house dust mite allergen was reduced. The control group followed standard advice. Children were assessed at ages 1, 2, 4, 8 and 18 years for the presence of asthma and atopy.

Results At 18 years of age, there was a significantly lower prevalence of asthma in the prevention group compared with the control group (OR: 0.23, 95% CI 0.08 to 0.70, p=0.01), primarily due to asthma that developed during childhood but persisted until age 18 years. Repeated-measure analysis showed that there was an overall reduction in asthma prevalence from 1 to 18 years (OR: 0.51, CI 0.32 to 0.81, p=0.04). Prevalence of atopy was not significantly different between the two groups at age 18.

Conclusion Comprehensive allergen avoidance in the first year of life is effective in preventing asthma onset in individuals considered at high risk due to heredity. The effect occurs in the early years, but persists through to adulthood.

Key messages

What is the key question?
- Can asthma be prevented by allergen avoidance during infancy?

What is the bottom line?
- A comprehensive allergen-avoidance regime in high-risk infants during infancy reduced asthma onset during childhood.

Why read on?
- This is the only primary prevention study of asthma, which shows a positive outcome throughout childhood.

INTRODUCTION

Over the last 50 years, the prevalence of asthma has increased dramatically, with an estimated global occurrence of 300 million.[1] Despite intensive efforts to develop novel therapeutic agents, asthma is still an incurable disease with pharmacotherapy at best achieving abeyance of symptoms. Research has highlighted the importance of the interaction of gene and environment, particularly in the early years of life.[2] Atopy is arguably the most significant single risk factor for asthma with a population-attributable risk of 56% in some, but not all populations.[3] Environmental factors represent an opportunity for intervention in asthma prevention.[4] House dust mite exposure (HDM),[5] dietary intake,[6] microbial[7] and viral exposures[8] are potential factors influencing asthma onset. Optimal timing of primary prevention is unknown, but early life immunological development in atopic infants,[9] and evidence of airway changes consistent with asthma in infants,[10] argues for early intervention within the first few months of life. Further, early sensitisation to HDM has been consistently associated with childhood asthma.[11]

We hypothesised that in infants genetically predisposed to atopy, allergen avoidance of both house dust mites and common food allergens during infancy may lead to a reduction in the development of allergic diseases with the benefit continuing beyond the actual period of avoidance. Consequently, 120 infants were recruited into this intervention study in 1990 and assessed at the ages of 1, 2, 4 and 8 years. Outcomes at these follow-ups have been reported previously and have shown significant reduction in asthma, eczema and atopy in the intervention group.[12–16] We now report the outcome of this randomised controlled trial examining the preventive effect of a multifaceted allergen avoidance strategy in the first year of life.

METHODS

Detailed descriptions of the intervention methodology have been published previously.[12] Briefly, in 1990, 120 infants who were considered at high risk of developing allergic disease on the basis of dual heredity (two or more immediate family members (parents and/or sibling) with an allergic disorder) or

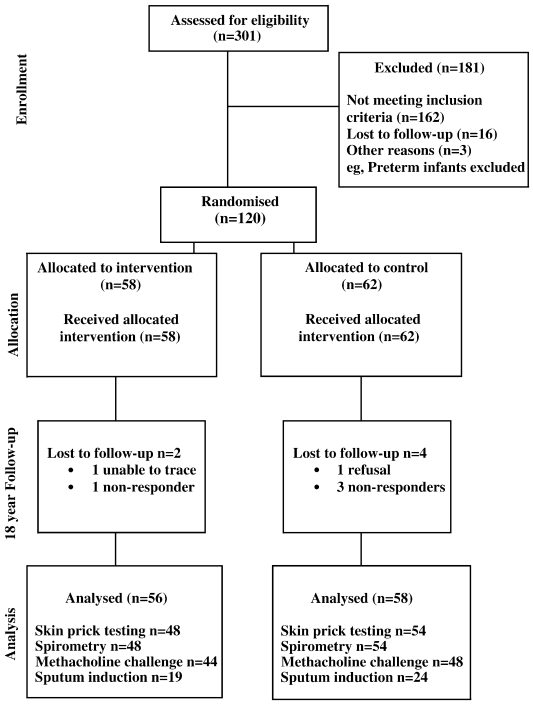

Figure 1 Consort diagram of the study.

single heredity (one parent or sibling with an allergic disorder) plus cord total IgE >0.5 kU/l were recruited at birth. Participants were randomised into a prevention group (n=58) and a control group (n=62) using computer-generated random allocation numbers.[12] The study was single-blinded, so that the participants' grouping was concealed from the researchers until after assessment at each follow-up. From birth until 12 months, lactating mothers and infants in the prevention arm followed a diet of strict avoidance of: dairy products, egg, soya, fish and shellfish, peanut and tree nuts. HDM reduction measures were taken, including vinyl mattress covers and use of acaricide in bedrooms and living rooms. Dust samples collected at 0 and 9 months showed that the prevention group had significantly lower levels of HDM than the control group (median Der p1 5.86 µg/g (interquartile range 3.55–10.93) vs 15.31 µg/g (7.25–27.49), p<0.0001). The control group was provided with standard recommendations prevalent at the time. A 100% follow-up was achieved at 1, 2, 4 and 8 years of age. Assessments consisted of parental questionnaires regarding symptoms of asthma and allergic diseases. Skin prick testing was performed in the majority of participants at each follow-up.[12–15]

Eighteen-year follow-up

The study was approved by Southampton & South West Hampshire Research Ethics Committee (07/HO504/188) and the protocol registered with ISRCTN (96472018). Of the original 120 participants, 114 (95%) gave informed consent for the assessment at age 18. The researchers assessing the participants were blinded to their group allocation. As in previous assessment, all participants answered the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire[17] and, where appropriate, reported the type of asthma medication used and answered the Juniper asthma-specific quality-of-life questionnaire.

For 103 participants (48 in the prevention group and 55 in the control group), allergy skin prick testing was performed by a standardised method[18] to house dust mite (*Dermatophagoides*

pteronyssinus), grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata, Cladosporium herbarum*, milk, hens' egg, wheat, soya, cod, peanut and in addition, histamine and physiological saline were used as the positive and negative controls, respectively (Alk-Abello, Horsholm, Denmark).

Exhaled nitric oxide (FeNO) was measured (Niox mino, Aerocrine AB, Solna, Sweden) according to ATS guidelines,[19] prior to spirometry. Baseline pulmonary function was measured using Koko spirometry software (PDS Instrumentation, Longmont, USA) according to standardised methodology.[20] Methacholine bronchial challenge was performed in accordance with international guidelines.[21] In order to compare bronchial hyperresponsiveness where participants did not achieve a 20% (or more) drop in their FEV_1, the dose response slope was calculated.[22] Induction, processing and analysis of sputum samples were undertaken in accordance with ERS guidelines.[23]

Definitions

A participant was defined as atopic where (s)he had at least one positive skin prick test to a food or aeroallergen. A skin prick test was defined as positive where the mean weal diameter was ≥3 mm larger than the negative control. Asthma was defined as a positive response to (1) has a doctor diagnosed you with asthma and, either (2) have you wheezed in the last 12 months or (3) are you on inhaled corticosteroids? Atopic asthma was defined by the combination of asthma plus atopy.

Table 1 Asthma, asthma phenotypes and atopy at 18-year follow-up

	Prevention (n=56)* n (%)	Control (n=58)* n (%)	p Value‡
Any current asthma	6 (10.7)	15 (25.9)	**0.04**
Persistent asthma	3 (5.4)	10 (17.2)	**0.04**
Late-onset asthma	3 (5.4)	5 (8.6)	0.38§
Remitted asthma	7 (12.5)	5 (8.6)	0.50
Ever had asthma	13 (23.2)	20 (34.5)	0.18
	Prevention (n=48)† n (%)	**Control (n=55)† n (%)**	**p Value‡**
Atopic asthma at 18	4 (8.3)	13 (23.6)	**0.04**
Non-atopic asthma at 18	2 (4.2)	2 (3.6)	0.64§
Any current atopy	21 (43.8)	28 (50.9)	0.47
Persistent atopy	9 (18.8)	22 (40.0)	0.19
Late-onset atopy	12 (25.0)	6 (10.9)	0.06
Remitted atopy	0 (0.0)	1 (1.8)	0.53§
Ever been atopic	21 (43.8)	29 (52.7)	0.36
Any current HDM sensitisation	14 (29.2)	23 (41.8)	0.18
Persistent HDM sensitisation	6 (12.5)	17 (30.9)	**0.02**
Late-onset HDM sensitisation	8 (16.7)	6 (10.9)	0.40
Remitted HDM sensitisation	0 (0.0)	1 (1.8)	0.53§
Ever been HDM sensitised	14 (29.2)	24 (43.6)	0.13
Any current food allergen sensitisation	9 (18.8)	8 (14.5)	0.57
Persistent food allergen sensitisation	0 (0.0)	1 (1.8)	0.53§
Late-onset food allergen sensitisation	9 (18.8	7 (12.7	0.40
Remitted food allergen sensitisation	3 (6.3)	7 (12.7)	0.22§
Ever been food allergen sensitised	12 (25.0)	15 (27.3)	0.79

The bold values in the last column (p value) indicate statistically significant differences.
*Base: all participants in 18-year follow-up.
†Base: participants who underwent SPT and spirometry.
‡p Values are from χ^2 tests, except those marked §which are from Fisher's exact tests.
Persistent, onset at/before 8 years follow-up and still current at 18; late-onset, onset between 8 and 18 years; remitted, onset at/before 8 years but not current at 18 years.

Asthma in childhood is variable in terms of onset, remission and relapse. To test whether the intervention was associated with the different types, we classified participants into one of the following groups: 'persistent asthma' (asthma onset at/before 8 years and current asthma at 18 years), 'late-onset asthma' (asthma at 18 years but no prior history of asthma), 'remitted asthma' (no asthma at 18 but prior history of asthma) or 'never asthma' (no current or prior history of asthma).

Statistical methods

Details of statistical methodology are provided in the on-line supplement. Briefly, an intention-to-treat analysis was performed using all available data. The sample size was limited to the original 120 participants who were recruited prenatally 18 years ago. The primary outcome of the current analysis was asthma at 18 years, and longitudinally from 1 to 18 years of age.

Depending on the significance in bivariate analysis, six explanatory variables were tested for inclusion in the binary regression model: (1) group, (2) dual heredity, (3) family history of asthma (at least one parent or sibling with asthma), (4) whether or not the subject was a firstborn child, (5) exposure to smoke in the 2 years preceding the 18-year follow-up and (6) maternal smoking during pregnancy.

To assess the relationship between groups and the different types of asthma (never asthma, persistent asthma, remitted asthma and late-onset asthma), a multinomial logistic regression model was built using the same model-building strategy as for the binary regression model. Longitudinal analysis was undertaken using generalised estimating equations (GEE) with a logit link function and an independent correlation structure. The GEE analysis was based on the 114 subjects who were followed-up at 18 years, as well as at age 1, 2, 4 and 8 (a total of 547 data points).

RESULTS

Asthma at 18 years: cross-sectional analysis

At the age of 18 years, 114 of 120 (95%) were assessed; 56/58 (96.6%) from the prevention group and 58/62 (93.5%) from the control group (figure 1). The prevalence of asthma was significantly lower in the prevention group compared with the control group (10.7% and 25.9%, respectively); the OR was 0.34, 95% CI 0.12 to 0.96, p=0.04) (table 1). The significantly lower prevalence of asthma at 18 years was due mainly to a lower prevalence of persistent asthma rather than late-onset asthma.

The binary logistic regression found that only two variables were significantly associated with asthma at 18 years once other explanatory variables were held constant: group and family history of asthma. The final model contained these two covariates. The odds of asthma at age 18 were 4.33 times greater if there was a family history of asthma (CI 1.37 to 13.74, p=0.01). Once family history of asthma was held constant, the odds of asthma at age 18 for the prevention group were 0.23 times the odds for the control group (CI 0.08 to 0.70, p=0.01). In other words, the effect of the intervention was stronger once we adjusted for family history of asthma. We tested for interaction between group and family history of asthma, but this was not significant (p=0.48).

The frequency and severity of asthma symptoms and asthma-specific quality-of-life scores[24] were not significantly different between the groups (table 2). Further, there was no significant difference in terms of lung function, bronchial hyperresponsiveness, FeNO or airways inflammatory cells.

Table 3 shows the results of the multinomial logistic regression model. The figures in the table are predicted probabilities,

Table 2 Symptoms, quality of life, lung function and airway inflammation in participants with asthma

Variable	Prevention (participants with asthma = 6)	Control (participants with asthma = 15)	p Value
Symptoms in the last 12 months			
Wheeze on exertion	6 (100.0%)	13 (86.7%)	1.00
Wheeze affecting speech	2 (33.3%)	5 (33.3%)	1.00
>4 wheeze attacks	4 (66.7%)	5 (33.3%)	0.36
Sleep affected >1 night a week	1 (16.7%)	2 (12.5%)	0.91
Asthma quality-of-life scores	5.06 (4.00–6.10)	6.01 (5.60–6.50)	0.07
FEV_1, % predicted	85.67 (17.39)	97.55 (12.96)	0.11
FVC, % predicted	91.80 (12.43)	96.50 (17.03)	0.55
FEV_1/FVC, % predicted	94.71 (16.23)	101.75 (7.95)	0.20
PEFR, % predicted	95.15 (21.77)	100.88 (17.24)	0.53
FeNO, ppb	21.38 (1.5)	33.86 (2.13)	0.10
Sputum eosinophils, %	3.0 (1.8–3.5)	1.8 (1.1–5.9)	1.00
Sputum neutrophils, %	9.5 (2.9–16.2)	5.7 (1.6–15.8)	0.47
Sputum epithelial cells, %	2.8 (1.15–6.8)	5.3 (2.9–9.9)	0.08

Numbers are frequencies (%), means (SD) or median (IQR).
p Values are Pearsons χ^2 (frequencies) or two-sample t test (means) except for quality of life and induced sputum results (Mann–Whitney U test). Six and 14 participants with asthma in the prevention and control groups underwent spirometry and FeNO (geometric mean and SD reported). Four and 10 of them in the prevention and control groups underwent methacholine challenge, and 4 and 7 were able to provide an induced sputum sample.
DRS, dose response slope; FeNO, exhaled nitric oxide.

that is, the proportion of the study participants in each group who would have each type of asthma according to the model (thus, each row sums to 100%). These results confirm that the association between the intervention and asthma at 18 is almost entirely due to the prevention group being significantly less likely to have persistent asthma; there was no significant association between group and late-onset or remitted asthma, even when family history of asthma was held constant. The predicted probability of persistent asthma among those with no family history of asthma was five times higher in the control group than in the prevention group (8.5% and 1.6%, respectively). Similarly, the predicted probability of persistent asthma among those with a family history of asthma was four times higher in the control group than in the prevention group (28.0% and 7.0%, respectively).

Atopy and asthma at 18 years: cross-sectional analysis

There were no significant differences in the prevalence of atopy, HDM sensitisation or food allergen sensitisation between the groups at age 18 years (table 1). Although atopy was not significantly different between the two groups at 18 years (OR: 0.75, CI 0.34 to 1.63, p=0.47), prevalence of atopic asthma was significantly lower in the prevention group (OR: 0.29, CI 0.09 to 0.97, p=0.04) (table 1). This finding led to the hypothesis that the intervention was acting by reducing the effect of atopy in inducing asthma. To test this hypothesis, a binary logistic regression model was run with the interaction between atopy and the group as the sole explanatory variable. The interaction term did not have a significant association with asthma at 18 years, which was unsurprising given the small numbers. The hypothesis is a plausible one, but a larger study would be required to test it properly.

Table 3 Results of multinomial logistic regression model: predicted probability of being in each 'asthma' group

	Predicted probability of:			
	Never asthma	Remitted asthma	Persistent asthma	Late-onset asthma
Prevention group, no family history of asthma	90.3%	6.1%	1.6%*	2.1%
Control group, no family history of asthma	80.2%	6.1%	8.5%*	5.2%
Prevention group, family history of asthma	70.9%	15.3%	7.0%*	6.8%
Control group, family history of asthma	47.5%	11.7%	28.0%*	12.9%

*p<0.05.

Asthma over childhood and adolescence: longitudinal analysis

GEEs assessed the association between the prevalence of asthma throughout the length of the study with repeated-measure analysis, adjusted for group, sex, dual heredity, firstborn status, maternal, paternal smoking and pet exposure. The prevention group was significantly less likely to have asthma throughout childhood (OR: 0.51, CI 0.32 to 0.81, p=0.04) (figure 2). Males was significantly associated with an increased risk of asthma within this model (OR 1.71, CI 1.11 to 2.6, p=0.02), and no other variable was significantly associated with an increased risk of asthma within this model.

Repeated-measure analysis showed that over the length of follow-up there was a significantly lower period of prevalence atopy in the prevention group compared with the control group (OR: 0.42, CI 0.25 to 0.83, p=0.007) (figure 3). The difference between the groups was noticeable from the earliest follow-up at 1 year, and persisted through to 8 years, when the difference had narrowed and became non-significant at 18 years (figure 3).

DISCUSSION

Our study demonstrates a significant and sustained reduction in the prevalence of asthma in participants who underwent a comprehensive food and house dust mite allergen-avoidance strategy in the first year of life. Atopy is a significant factor in the development of asthma. Our analysis demonstrates a significant reduction in the prevalence of atopy and, specifically, HDM sensitisation in the prevention group in early childhood (table 1), resulting in a significant reduction in persistent asthma, and therefore, the difference in asthma prevalence still being significant at 18 years, specifically atopic asthma. There was no significant effect on atopy or asthma developing during adolescence. However, the longitudinal analysis demonstrates an overall significant difference in the prevalence of both asthma and atopy over the duration of follow-up (figures 2 and 3). Further follow-up will determine whether our intervention has successfully prevented the onset of asthma or merely delayed it to later adult life.

The study has several limitations. The number of randomised participants was small, and so the study may not have had adequate power for all the variables tested. However, the finding of a significant difference in the primary outcome measure of asthma prevalence is a valid one, as power is only critical to prevent Type II error where the null hypothesis, of no significant difference between the two groups, is inappropriately accepted. Another limitation is that participants were aware of the group allocation and, hence, reporting bias remains a possibility. However, preventive effect has been consistent throughout 18 years, including asthma treatment prescribed by their physicians. In view of the open design, the control group may have taken measures to reduce allergen level, but that would serve to reduce the difference between the groups. The active intervention stopped at 12 months; however, it is possible that parents in the

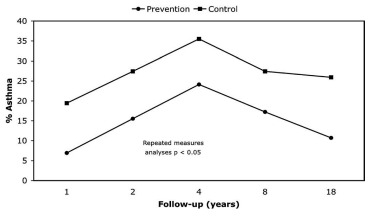

Figure 2 Prevalence of asthma over 18 years of follow-up. Generalised estimating equation-repeated measures analysis, adjusted for first born status, dual heredity, exposure to paternal and maternal smoking and pets in the home.

prevention group may have continued with some allergen-avoidance measures. There was concern regarding an under-reporting of asthma symptoms by adolescents in the intervention group if they were aware of their group allocation. We asked 23 randomly selected participants regarding their group allocation. Only three could correctly identify their group. Thus, we do not think that response bias influenced the outcome of this study.

The two groups were matched in terms of education, smoking and participation in this study (table E1). While there were significantly more participants with dual heredity in the prevention group and firstborns in the control group, neither of these variables influenced the outcome in either cross-sectional or longitudinal analysis. We did not adjust for multiple testing as the primary outcome variables, that is asthma and atopy, were determined a priori, before this analysis, at the inception of this RCT, and subsequently at every follow-up.[12–16] Symptoms, lung function and markers of airway inflammation did not significantly differ between patients with asthma in the prevention and control groups (table 2). However, the numbers were small and the possibility of type II error (false negative) cannot be excluded. All we can say is that the intervention reduced the prevalence of asthma globally rather than preventing onset at the milder or severe end of the asthma spectrum.

Our finding of a significant reduction in asthma using the dual intervention of HDM avoidance and dietary modification is unique in terms of the comprehensive allergen-avoidance regime, the overall length of follow-up and the size of the preventive

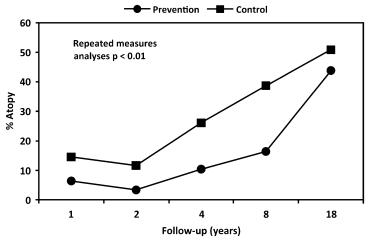

Figure 3 Prevalence of atopy over 18 years of follow-up. Generalised estimating equation-repeated measures, bivariate analysis.

effect observed. Other studies using similar, although not the same, multifaceted intervention had some success. The Prevention of Asthma in Children study found that allergen avoidance was associated with a significant reduction in parental reporting of asthma symptoms at 2 years.[25] The Canadian Childhood Asthma Primary Prevention Study at their 7-year follow-up found a significant reduction in the prevalence of asthma in the intervention group compared with their control group (14.9% vs 23%).[26] The requirement for the combination of dietary modification in addition to house dust mite avoidance and the additional need for prolonged breast feeding/delayed introduction of allergens into the infants' diet may account for the lack of success of single-intervention trials of house dust mite avoidance,[27 28] or dietary modification,[29] to significantly reduce the prevalence of asthma. Manchester Asthma and Allergy Study is the epitome of single-allergen intervention where extensive mite allergen-avoidance measures resulted in reducing HDM levels to very low levels, and yet, failed to prevent asthma.[30] This notion is supported by a systematic review, which concluded that multifaceted interventions were effective, at least to some extent, in reducing the development of asthma while single interventions failed to have any effect.[31]

Recent epidemiological observations support the notion of immune tolerance induction, rather than allergic sensitisation following early high-dose exposure to allergen.[32] So how can we reconcile these observations with the current study where a reduction in allergen exposure seems to be protective? There may be a number of explanations. Most of the data on high-dose exposure being protective comes from foods such as peanut, rather than inhalant exposure.[32] Thus, the nature of allergen and route of exposure may be important in determining the outcome.[33] Further, there may be a non-linear relationship between allergen exposure and sensitisation so that very low and high exposures may lead to tolerance, while a moderate level, and/or repeated exposure, may cause sensitisation.[34] Genetics may offer yet another explanation for our findings. The effect of environmental exposure may depend on the genetic sequence variation,[35] with opposite outcomes possible following exposure to the same exposure.[36] Although, the island population is not inbred, it is possible that participants in this study were particularly responsive to the effect of a comprehensive reduction in allergen exposure. A larger, multicentre trial with information on genetic and epigenetic features may answer some of these critical questions.

This is the only study that has shown a persistent and significant reduction in asthma and atopy throughout childhood. Other studies have not shown these benefits, but none of the other trials have replicated the design and methodology of this trial. For a number of reasons, which include small sample sizes and genetic homogeneity, the prevention effect seen in this study may not be generalisable. However, given the heterogeneous nature of asthma, it is unlikely that a single intervention of any kind would be effective in all participants. The significance of asthma prevention is such that further studies are warranted to identify the subgroups where reduction in allergen exposure might be effective.

In conclusion, our study provides evidence that a combined dietary and environmental allergen-avoidance strategy in the first year of life is successful as a primary prevention strategy for asthma in high-risk individuals, with benefits persisting into early adulthood. There is an urgent need to replicate these findings in a large multicentre study with stratification to investigate effectiveness and cost-effectiveness, and the immunological mechanisms underlying this approach.

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Contributors SHA conceived the idea and worked with all the authors on the plan for recruitment, assessments and analysis, and reviewed and revised draft. MS undertook the recruitment and assessments, performed data entry and initial statistical analysis and wrote the first draft. AN reviewed and revised the statistical analysis. GR, SM and RK helped with the draft revisions. All the authors reviewed and discussed the results, contributed to the manuscript and approved the final version.

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Competing interests None.

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DECLARATION OF AUTHORSHIP

I, Martha Scott

declare that this thesis, **Primary prevention of asthma and associated allergic disease**, and the work presented in it are my own and has been generated by me as the result of my own original research

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

   Multifaceted allergen avoidance during infancy reduces asthma during childhood with the effect persisting until age 18 years
   M Scott, G Roberts, R J Kurukulaaratchy, S Matthews, A Nove, S H Arshad
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Signed: ..... Martha Scott

Date: ..... 31 December 2013

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Dedication

This thesis is dedicated to my Grandma Brook, who taught me to look beyond the obvious.

Definitions and Abbreviations

BHR:	Bronchial hyperresponsiveness
BTS:	British Thoracic Society
DRS:	The % decline in FEV_1 from the post- saline value to the final dose of methacholine that was administered divided by the cumulative dose of methacholine administered
FeNO:	Exhaled nitric oxide
FEV_1:	Volume of air expired forcibly in one second
FVC:	Vital capacity forcibly expired
FEV_1/FVC:	Ratio of FEV_1 divided by FVC
GEE:	Generalised estimating equations
HDM:	House dust mite
PC_{20}:	The PC20 is that concentration of methacholine, which causes a 20% drop in FEV1.
SPT:	Skin prick test

Chapter 1: Primary prevention of asthma and associated allergic disease: a literature review

1.1 The prevalence and burden of asthma

Over the last 50 years multi-centre studies have confirmed a worldwide increase in the prevalence of allergic disease[1],[2]. The global prevalence of asthma is estimated to be 300 million[3] with an estimated 50% increase over each decade[4]. In the UK the prevalence of asthma in adults aged 16-44 years of age is 4.4 %[5], in adults aged over 65 the prevalence of untreated asthma is 1.7%[6]. In children aged 10 the prevalence of current asthma is 13% [7].

Asthma is estimated to account for an estimated 1 in every 250 deaths worldwide[8]. The UK has one of the highest prevalence's of asthma in the world with 1 in every 7 children aged 2 to 15 years and 1 in every 25 adults, having asthma symptoms requiring treatment[9]. The economic costs of asthma are high. Asthma care and related services cost the NHS almost £900 million, and asthma is responsible for at least 12.7 million lost working days every year[10]. The cost to the individual with asthma is considerable with one in four asthmatic subjects reporting productivity losses, leisure time losses or use of hospital services because of asthma[11]. Severity of asthma is correlated with reduced quality of life scores[12] and worsening mental health, specifically with depression[13]. Lung function (FEV_1) has been shown to correlate with asthma with a higher disease burden associated with decreasing lung function (FEV_1)[11].

1.2 Defining Asthma

Our current concepts and understanding of asthma are built on the observations made over two millennia. The term asthma is derived from the Greek word *azeein* meaning sharp breath. Araetaeus a Greek physician (Second century AD) provides one of the earliest descriptions of asthma:

'heaviness of the chest; sluggishness to one's accustomed work, and to every other exertion; difficulty of breathing in running or on a steep road; they are hoarse and troubled with cough[14]'.

In his 'Treatise on Asthma; Its Pathology and Treatment' published in 1860, Henry Hyde Salter, a London physician, described the disorder as:

'paroxysmal dyspnoea of a peculiar character, generally periodic with intervals of healthy respiration between the attacks'[15].

Over a 120 years later the American Thoracic Society in 1986 echoed Dr Salter defining asthma as

'a clinical syndrome characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli. The major symptoms of asthma are paroxysms of dyspnea, wheezing, and cough, which may vary from mild and almost undetectable to severe and unremitting (status asthmaticus). The primary physiological manifestation of this hyperresponsiveness is variable airways obstruction.'[16]

The difficulty with such symptom-based definitions is that our lungs have finite responses in terms of symptoms and signs in response to pathophysiological processes. Hence a functional definition of asthma carries the risk that conditions that are appreciably distinct and discrete from one another are brought together under the umbrella term 'asthma'.

In 2007 the Global Initiative for Asthma (GINA) defined asthma as:

'a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment'[17].

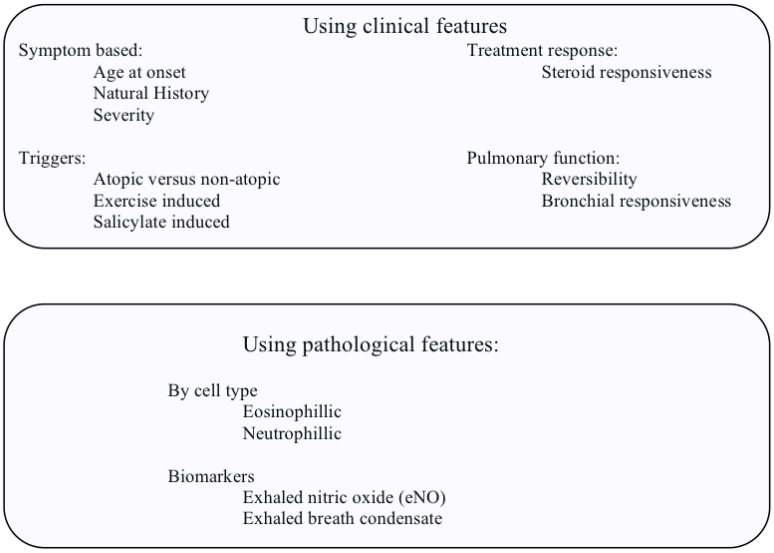
The GINA definition is a pragmatic one, what we think of as asthma in terms of a syndrome of signs and symptoms is the final pathway of many different and possibly discrete processes.

1.3 The heterogeneity of asthma

The move from research focusing on asthma as a single entity with variable expression to an increasing awareness that different subgroups of asthma exist in terms of pathogenesis, natural history and response to therapeutic intervention has developed over the last 60 years. Rackmann in 1947 classified asthma into two distinct subgroups of asthma: 1) extrinsic (allergy related) and 2) intrinsic (non-allergy related) asthma[18]. Over the next 50 years research focused almost exclusively on the association between atopy, allergy and asthma[19] as well as

identifying risk factors for asthma severity[20]. In the last 20 years however the heterogeneity of asthma has attracted more mainstream research interest. Research has focused on categorising asthma by various methods as both epidemiological and pharmacotherapy orientated research has highlighted that differing subgroups of asthma are clinically significant (**Figure 1.1**). Epidemiologically childhood longitudinal studies have demonstrated that childhood asthma/wheeze shows considerable heterogeneity in terms of time of onset, with persistent, early and late onset phenotypes being identified[21]. In addition childhood asthma may remit, and then relapse in later life[22]. Onset at different time points are associated with different levels of severity[23]. Pharmacotherapy orientated research has identified clinically important differences in terms of how different subgroups respond to treatment, for example non eosinophilic asthma is associated with a poor response to steroids[24].

Figure 1.1. Methods of classifying asthma phenotypes



1.4 The association between asthma, atopy and allergic disease

Atopy describes the phenomenon of genetically predisposed individuals producing IgE antibodies in response to exposure to ordinary amounts of allergens. These allergens are generally, but not exclusively, proteins that are common within our environment[25], and to which the majority of non-predisposed individuals fail to demonstrate symptomatic reactions. The diagnosis of atopy requires evidence of IgE sensitization – for example by positive skin prick testing to allergens or by specific IgE antibodies present in the serum. Atopy is common in the general population with studies demonstrating a prevalence of 26.9%[26] to52% [27] in un-selected populations.

Allergy is a specific type of hypersensitivity reaction where the symptoms and signs initiated by exposure to a specific stimulus (allergen) are mediated by IgE antibodies.

Allergic diseases represent a particular organ or mucosal surfaces response to allergen driven inflammation, and require the presence of atopy to be designated as of allergic origin. Asthma, rhinitis and eczema are examples of diseases that in the presence of atopy are considered to be allergic diseases.

Epidemiological work shows that there is a strong association between asthma, and atopic diseases such as rhinitis and eczema. The presence of one atopic disease increases the risk of developing another, with one study estimating the odds ratio ranging from 2.4-3.4[28]. Asthma and rhinitis are closely associated with up to 70% of people with asthma reporting co-existent rhinitis[4]. Longitudinal studies have found that

atopic rhinitis is a significant risk factor for developing asthma, doubling the risk of onset[29]. Adults diagnosed with both asthma and allergic rhinitis experienced more asthma-related hospitalizations and GP visits, and incurred higher asthma drug costs than adults with asthma alone[30]. Eczema is also closely associated with asthma between 15-30% of eczema suffers having co-existent asthma[31] as well as a increased risk of asthma onset with one systemic review finding the pooled odds ratio for the risk of asthma after eczema, compared with children without eczema was 2.14 (95% CI, 1.67-2.75)[32]. Similarly to rhinitis the presence of eczema is associated with a more severe asthma burden[31].

1.5 Is all asthma allergic?

Undoubtedly asthma and atopy are closely associated. A recent study found that the prevalence of atopy in asthmatics was 79% versus 54% for the general population, with a population attributable risk of atopy for asthma of 56%[27]. Indeed one may argue that atopy is the single biggest risk factor for asthma onset. Until relatively recently asthma was considered predominantly as an allergic disease. Researchers are however increasingly aware that non-atopic asthma, whilst less common than atopic asthma, is a discrete entity in its own right and appears to differ from atopic forms in terms of aetiology, risk factors, natural history, and response to pharmacotherapy[24].

1.6 The Pathogenesis of asthma

1.6.1 The airways in asthma

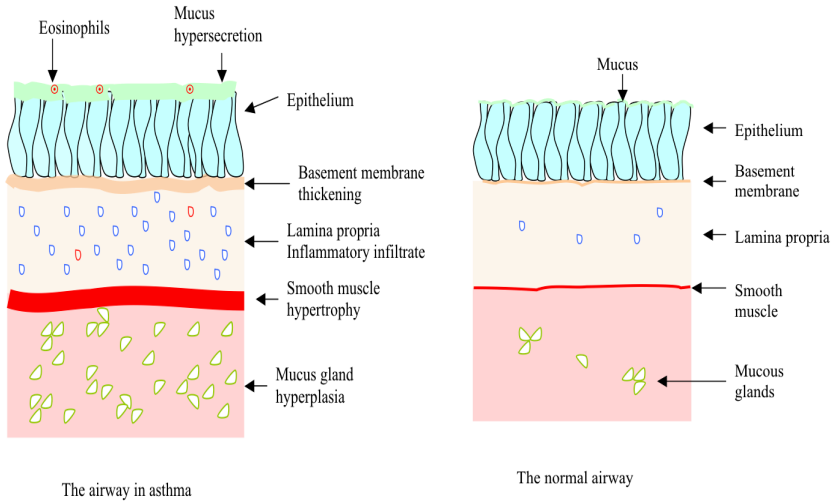
Osler, in 1901, in his review of the aetiology of asthma[33] noted

'*That in many cases it is a special form of inflammation of the smaller bronchioles'.*

With the advent of flexible bronchoscopy in the 1980's, bronchial biopsies and induced sputum consistently found differences between the airways in asthma compared to non-asthmatic airway (**Fig. 1.2**). The main differences being: increased numbers of goblet cells in the airway epithelia, thickening of the layer beneath the basement membrane leading to sub-epithelial fibrosis, inflammatory infiltrates of mast cells, neutrophils and eosinophils in the lamina propria, smooth muscle hypertrophy and increased size of sub mucosal glands[34-36].

The end result of all these changes is airways narrowing giving rise to the symptoms and physiological changes of asthma. The next question is what leads to these changes?

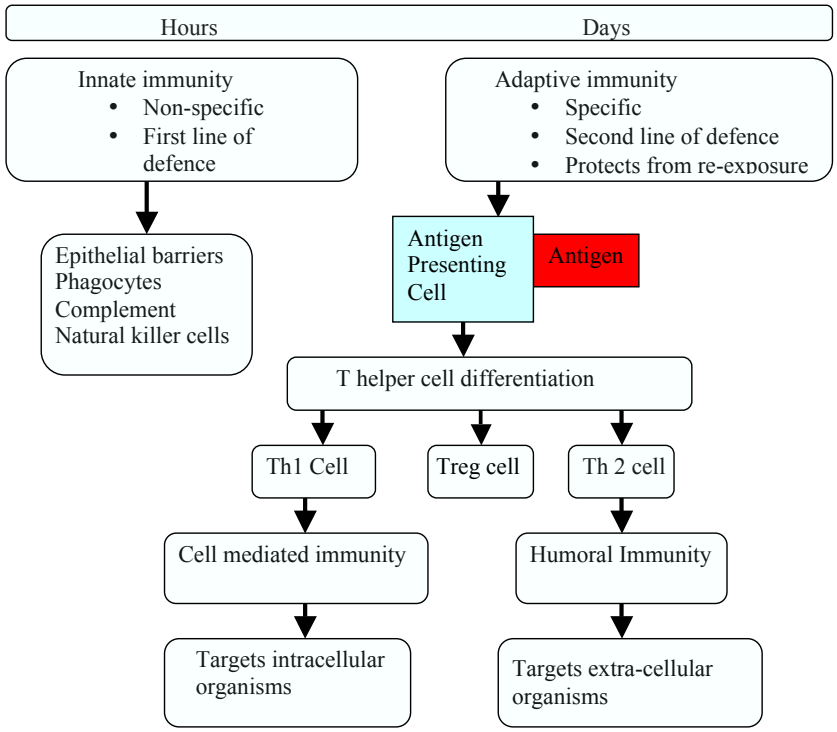
Fig 1.2. Differences between the asthmatic and non-asthmatic airway



1.6.2 The Th2 Hypothesis

The immune system is complex, composed of different, but interlinking pathways jointly providing an immediate as well as a delayed response to a perceived threat (Fig 1.3). Inflammation is a key part of the immune systems defence against pathogens but when it becomes dysregulated or skewed then allergy arises

Fig 1.3 Overview of the immune system

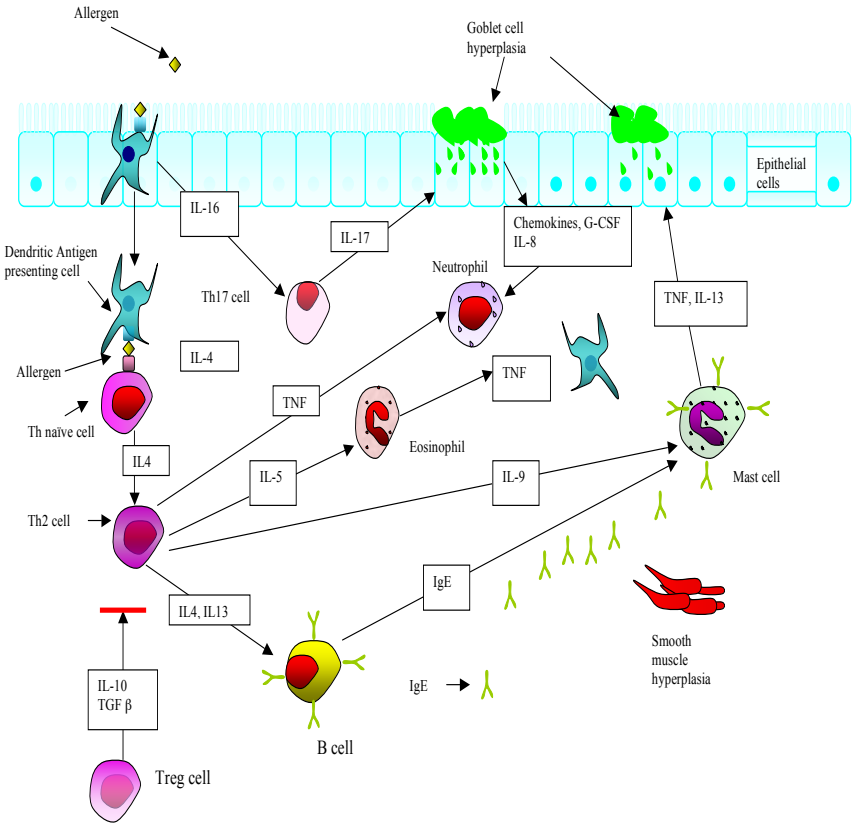


Th cells act as 'managers' co-ordinating and mobilizing other cells and pathways involved in immune defence. When antigen-presenting cells (APCs) present antigen to naïve T cells this stimulates the T cells to differentiate into either Th1 or Th2 cells. A third type of T lymphocyte, the regulatory T cells (Treg), limits and suppresses the immune system, and may control aberrant immune responses to self-antigens; an important mechanism in controlling the development of autoimmune disease and suppressing allergy.

Work identifying CD4 Th subsets and their functional effects including cytokine release (**Table 1.1**)[37] along with studies demonstrating that Th2 cells are a persistent feature of the asthmatic airway [38] led to the theory that Th2 cells are an important mechanism in promoting asthma. According to the Th2 hypothesis, inflammation occurs in the asthmatic airway because the immune system becomes skewed towards a Th2 response (**Figure 1.4**.) leading to excess Th2 derived cytokines promoting airways inflammation[39]. Studies investigating the effects of aeroallergen induced inflammation in sensitized individuals provides support for this finding that aeroallergen exposure is associated with increased markers of Th2 driven airways inflammation[40].

The mechanisms by which this occurs are not fully known but both innate and adaptive immunity play a role. More recently the Th2 hypothesis has been extended as the importance of regulatory T cells in controlling the immune response has been recognised. Increasingly disregulation of Th cells with impaired regulatory responses of T reg cells are seen as an important feature of asthma pathogenesis and cytokine release[41] .

Fig 1.4. Overview of the Th2 hypothesis in asthma



After antigen capture antigen-presenting dendritic cells present allergic peptides to naïve T cells, promoting their activation and differentiation into Th2 cells. Th2 cells produce cytokines, which mediate airway eosinophil and mast-cell recruitment, B-cell IgE isotype class switching, and mucus secretion.
Dendritic cells also produce IL-16 which as well as promoting Th2 differentiation triggers Th17 cell differentiation. IL-17 produced by Th17 cells mediates airway neutrophila and mucin production. T-reg cells negatively regulate the immune response to allergy, but their influence is attenuated in asthma.

Table 1.1: Principle cytokines and their effects within the lung

Cytokines		Main effect
IL-4	Th2	↑ IgE production ↑ number of Th2 cells
IL-5	Th2	↑ eosinophils
IL-6	Th2	↑ inflammation
IL-9	Th2	↑ mast cells
IL-12	Th1	↑ Th1 cells
IL-13	Th2	↑IgE production, induces airway remodelling
IL-17	Th1	↑ neutrophils
IL-18	Th2	↑ IFN-γ release
IL-25	Th2	↑ Th2 cells

Note: references[42], [43]

1.6.3 Similarities and differences between allergic and non-allergic asthma.

Studies comparing airway changes in atopic and non-atopic forms of asthma have, in the main, found no significant differences in terms of epithelial shedding, thickening of the basement membrane, smooth muscle hypertrophy, and interleukin expression[44-46] suggesting commonality in at least some of the pathways and mechanisms of atopic and non-atopic asthma. Research has highlighted that there is evidence of discrete differences in the degree of airway eosinophilia and neutrophilia. Clinically these subtypes appear to differ in their response

to pharmacotherapy, and in their natural history. For example the neutrophilic form is associated with more severe asthma.[47–50] This suggests that additional and/or alternative mechanisms may be involved in non-atopic asthma. These mechanisms are unknown but environmental factors such as early life infections, tobacco smoke exposure[51] as well as defects in airway barrier function[52] are considered to be important drivers of non-atopic asthma pathogenesis more so than in asthma associated with atopy where allergen driven inflammation is considered to be a key feature.

1.7 Airway remodelling and asthma and bronchial hyper-responsiveness

Histologically the hallmark of asthma is considered to be airway remodelling where there are structural changes in the small and large airways. These changes include increased smooth muscle mass, goblet cell metaplasia and mucus hypersecretion, sub epithelial fibrosis and abnormalities of the epithelium associated with increased shedding of the epithelial layer. Airways remodelling is considered to be driven by chronic airways inflammation whilst the precise mechanisms are unclear, these changes are considered to result in increased airway hyper-responsiveness and hence give rise to airflow obstruction[53–55].

Airway hyperresponsiveness (AHR) is an exaggerated physiological response of airways narrowing to environmental stimuli, for example allergens, exercise or cold temperatures, which is not seen in non-predisposed individuals[56].

AHR is measured using bronchoconstrictors, these are classified into two categories ‘direct bronchoconstrictors’ such as methacholine and histamine that act directly on smooth muscle and, ‘indirect’ which lead

to bronchoconstriction by causing the release of inflammatory mediators and/or via stimulating neural pathways[57] for example, AMP, hypertonic saline and exercise. Methacholine challenges are well standardised and are currently the most commonly used in clinical and epidemiological studies. A positive response is defined by a provocative concentration leading to a 20% fall in FEV_1 of less than 8mg/ml[58].

AHR to histamine or methacholine is found in 16-30% of children[59] and 10-16% of adults in the general population.[60]. The differences in prevalence of AHR between childhood and adulthood highlight that AHR is not a fixed sign, rather it can come and go over time. One study found that over an 18 year follow-up AHR 48% of participants changed their response status to a histamine challenge[61].

In subpopulations with asthma the prevalence of AHR is markedly higher occurring in 48-58%[60,62]. The exact nature of the relationship between AHR and asthma is complex. Whilst some studies show that asymptomatic AHR (AHR in the absence of respiratory symptoms) is a significant risk factor for asthma[63–65], suggesting that asymptomatic AHR may be an early pre-symptomatic stage in asthma pathogenesis; other studies have not found asymptomatic AHR to be a significant risk factor for asthma in later life[66–68]. Whilst AHR is far more common in asthmatic versus non-asthmatic populations there are still a large proportion of asthmatics who do not demonstrate significant AHR, one study finding that 42% of participants with current asthma symptoms had a negative bronchial challenge[59].

Further complications lie in the relationship between atopy and AHR. Atopic status has been found to be a significant risk factor for AHR with a higher prevalence in atopics 33% versus non-atopics 10.3% in the general population[69].

It is interesting to see how AHR has been utilised in studies of asthma over the last 30 years, in older studies the presence of AHR was considered to be crucial in diagnosing asthma. More recently

comparisons of AHR versus questionnaires to diagnose asthma (using physician diagnosed asthma as the 'gold standard') have found questionnaires to be equivalent[70] or superior in diagnosing asthma[56].

In conclusion AHR like asthma and atopy is a discrete entity in its own right. However AHR is far more likely to occur in the presence of atopy, and/or asthma. This suggests that AHR shares some common genetic factors with both atopy and asthma, but the presence or absence of AHR is not crucial to a diagnosis of asthma.

1.8 The Genetics of asthma

There is a strong association between a family history of asthma and an increased risk of asthma onset. Twin studies have found that there is greater concordance of asthma in monozygotic twins compared to dizygotic twins[71] with the heritability of asthma being between 60-75%[72,73]. Over the last three decades there have been an ever increasing number of genes that have been associated with asthma onset (**Table 1.2**).

Broadly these can be divided into four main groups of genes:

1 - Genes associated with innate immunity and immunoregulation

2 - Genes associated with T helper 2 cell differentiation and effector functions

3 - Genes associated with epithelial biology and mucosal integrity

4 - Genes associated with lung function, airway remodelling and disease severity

Table 1.2. Genes associated with asthma

Gene	Chromosome	Function
GSTM1	1p13.3	Environmental and oxidative stress — detoxification
Fillagrin	1q21.3	Epithelial barrier integrity
IL10	1q31-q32	Immunoregulation
CTLA4	2q33	T-cell-response inhibition and immunoregulation
IL13	5q31	TH2 effector functions
IL4	5q31.1	TH2 differentiation and IgE induction
IL18	11q22.2-q22.3	Induction of IFN and TNF
CD14	5q31.1	Innate immunity — microbial recognition
SPINK5	5q32	Epithelial serine protease inhibitor
ADRB2	5q31-q32	Bronchial smooth-muscle relaxation
HAVCR1	5q33.2	T-cell-response regulation — HAV receptor
LTC4S	5q35	Cysteinyl leukotriene biosynthesis — inflammation
LTA	6p21.3	Inflammation
TNF	6p21.3	Inflammation
HLA-DRB1, HLA-DQB1, HLA-DPB1	6p21	Antigen presentation
FCERIB	11q13	High-affinity Fc receptor for IgE
STAT6	12q13	IL-4 and IL-13 signalling
ADAM33	20p13	Cell–cell and cell–matrix interactions

References: [74], [75]

1.8.1 Genetics of atopy and asthma

Asthma and atopy have been repeatedly found to be closely associated with each other, with twin studies finding that asthma in one twin and atopy in the other (cross trait concordance) is greater for monozygotic than dizygotic twins[76] implying that there are genetic factors common to both asthma and allergic traits. Gene studies support this close association, for example chromosome 5q31-33 includes 14 genes that are associated with both asthma and atopy[74]. In certain circumstances there is evidence that both atopic and asthma genetic predisposition are vital for asthma expression. Filaggrin, a gene vital to maintaining the epithelial barrier, is an excellent example of the close ties between atopy and asthma. Filaggrin is found in the oral, nasal but not bronchial mucosa and is only associated with asthma in the presence of atopic dermatitis[77] suggesting that in these circumstances, asthma arises secondary to allergic sensitization occurring where the integrity of the epithelium is impaired.

1.8.2 Gene-Gene interactions

The combination of multiple genes involved in asthma as well as genes common to both asthma and atopy lead to complex interactions where a particular gene (and its variants) may have modest effects in isolation but when combined with other genes (and its variants) may synergistically impact on the risk of asthma and atopy. For example, Kabesch et al[75] found that the risk of atopy increased by 10.8 fold and asthma by 16.8 fold when polymorphisms in IL4, IL13, STAT6 and IL4A were combined stepwise compared with the effect of any single-nucleotide polymorphism.

Thus genes associated with atopy may interact with genes associated with asthma to increase the risk of the expression of asthma and atopy, beyond the risk associated with single polymorphisms in individual genes.

1.8.3 Gene – Environment interactions

Until relatively recently the impact of environmental factors on genetic expression was not considered to be particularly important. That view has changed with the growing recognition that genes as well as interacting with each other are also influenced by environmental exposure. Indeed environmental factors are considered to account for a significant proportion of between 20-40% [71] of the expression of asthma and for up to 50% of the expression of allergy [78]. The finding that high endotoxin exposure amongst children from farming communities is associated with a reduced prevalence of atopic asthma, hay fever and atopic sensitization, but not non-atopic asthma[79] has been further clarified by examining polymorphisms in TLR4 and TLR2 amongst children from farming communities (high endotoxin exposure) versus rurally based children from non farming communities. Farmers' children with a T allele in TLR2 versus non-farmers' children were significantly less likely to have asthma 3% versus 13% (both atopic and non atopic), atopy 14% versus 27%, and hay fever 3% versus 14%. When the results were stratified by high versus low endotoxin exposure, a variation in TLR2 was associated with a higher prevalence of atopy in the high endotoxin group[80]. The implications from studies such as these are that firstly environmental exposure plays a significant role in the expression of asthma and atopic disease. Secondly asthma and atopy are genetically closely associated. Thirdly despite the strong association between atopy and asthma in certain circumstances different genetic

and environmental mechanisms interact to lead to the expression, or surpression of asthma and atopic disease.

1.8.4 Asthma, a complex genetic disease

Clearly asthma is a complex disease – it does not follow classic mendelian pathways (a disease caused by a single gene mutation) rather it is polygenic with multiple genes, associated with asthma susceptibility. These genes may act directly by effects on barrier function, cytokine release, or indirectly by interacting with genes associated with atopy and bronchial hyperresponsiveness, or by a combination of direct and indirect pathways. Environmental factors add a further layer of complexity with a varied number of factors promoting or protecting individuals from asthma onset by interacting with genes associated with asthma, atopy and bronchial hyperresponsiveness. The precise mechanisms of how genes associated with asthma and atopy interact with each other, and with environmental factors to promote asthma onset are currently beyond our understanding. We can however conclude that whilst the genetics of asthma and atopy are independent of each other they can and do interact leading to asthma onset.

1.9 Phenotyping asthma

As our understanding that asthma arises from a complex interplay of multiple gene-gene interactions as well as gene-environmental factors the term phenotype ('the set of observable characteristics of an individual resulting from the interaction of its genotype with its environment') has started to be used to describe the different subgroups of asthma. Whilst there is still a long way to go in fully understanding the precise nature of and mechanisms involved in the interactions between genes and also with the individuals environment

the term phenotype at the least reminds us of the complexity involved and drives us on to elucidate the biological pathways involved in producing a particular phenotype.

1.10 Early life origins of asthma

Longitudinal studies have demonstrated that the majority of asthma in later life is preceded by asthma/wheeze in childhood[81-83]. Prospective studies have consistently found an association between childhood onset asthma and reduced lung function in asthmatics in adult life[82,84]. Studies tracking lung function measured in the first month of life found a significant relationship between reduced lung function in infancy and asthma/persistent wheeze at 10/11 years[85,86]. Increasingly evidence accumulates that airway remodelling in asthma begins in early life, with studies finding structural changes in symptomatic children less than 6 years of age[44,87]. Other studies have found that the airways of children without airway symptoms who then go on to develop asthma differ significantly from age matched controls, demonstrating that in children who develop asthma structural changes may predate symptoms by months or years[88]. In terms of timing of onset of airway changes, Sagliani et al found no significant changes in children with a median age of 1, but found by 3 there were significant changes in the airways[89,90]. The implication is that effective intervention needs to take place in infancy, specifically in the first year of life, in order to prevent the onset of airway remodelling and decline in lung function in the future. Further support for this view comes from studies such as Kuehni[91] who found that South Asian women who migrated into the UK >5years of age had a significantly lower prevalence of asthma than South Asian women who were either born in the UK or migrated <5years of age – 6.5% versus 16%. This

shows that it is early life environmental factors that influence the onset of asthma.

1.11 Why has the prevalence of asthma increased?

1.11.1 The Hygiene hypothesis

In 1989 Strachan found that there was a strong inverse association between family size, birth order and allergic rhinitis and eczema[92]. The relationship between asthma sibling position and family size was, whilst present, less strong[93]. Strachan suggested that an explanation for these findings could be that infections in early childhood transmitted by unhygienic contact with older siblings may prevent the onset of allergic diseases. This protective effect would be attenuated by decreasing family size as well as increasing standards of personal hygiene and would perhaps account for the rising prevalence in rhinitis, eczema and asthma. Whilst in a later analysis of a British longitudinal study followed from birth to 33, Strachan noted that whilst there was a significant positive association between rhinitis, eczema and small sibship and higher socio-economic status there was no association between these factors and asthma [94] suggesting that asthma may differ from rhinitis and eczema in terms of epidemiological risk[92]. Nonetheless Strachans seminal work highlighted the potential of environmental factors to influence the natural history of asthma and over the next 20 years epidemiological research has explored the influence and associations between environmental factors and asthma onset.

1.12 Risk factors for asthma and allergic disease

Epidemiological studies have identified numerous factors associated with asthma and allergic disease. Whether these associations are protective, or increase the risk, can often be difficult to establish as timing of exposure appears to be an important factor as to whether exposure will be protective or pathogenic. In addition environmental factors may act directly, for example by reducing asthma onset or more indirectly, by reducing the risk of atopy/other allergic diseases. A further layer of complexity is that these factors may interact with each other as well as with an individual's genetic risk.

1.12.1 Socio-economic status and asthma

The association between socio-economic status and asthma prevalence is less than clear. Broadly, global prevalence studies of asthma have found that countries (and regions) of high affluence have a higher prevalence of asthma than less affluent countries[95] or regions[96] . In addition rapid changes in affluence have been associated with increasing prevalence of atopy[97]. Within western countries the association between socio-economic status and asthma prevalence is more opaque. Some studies have found an association between socio-economic status and asthma – with lower socio-economic status associated with increased prevalence of asthma irrespective of atopic status[98]. In contrast, other studies have found that lower socio-economic status is associated with an increased prevalence of non-atopic asthma[99]. Studies assessing the impact of socio-economic status in less affluent countries have confirmed a significant inverse association between asthma prevalence and lower socio-economic

groups[100,101] whilst this has not been replicated in more affluent countries. It is plausible that the differences in exposure to allergens and microbials are likely to be more extreme within low affluent countries compared to within more affluent countries. Suggesting that there is a threshold effect to antimicrobials influencing atopy and hence asthma.

1.12.2 Animal exposure

Numerous studies have found a protective effect between a farming lifestyle and asthma, atopy and allergic disease[102–104]. Suggesting that higher exposure to microbes and allergens with farming communities is an important factor. This protective effect may be a double edged sword. Eduard et al found that in adult farmers whilst endotoxin exposure was associated with less atopic asthma there was an increased association with nonatopic asthma[105]. Portengen et al found significantly less atopic sensitisation amongst pig farmers but a higher prevalence of AHR amongst those who were sensitised[106].

Looking at studies in children the effect of microbial exposures is also complex. CD14 encodes for a receptor that interacts with the endotoxin from gram-negative bacteria; a study looked at the effects of 1) concentration of house dust endotoxin and 2) animal contact with groups divided into a) no animal contact, b) contact with dogs/cats and c) stable animal contact, on different alleles of the CD14 gene CC, TC, and TT[107]. Specific IgE was significantly lower in the CC allele with exposure to high concentrations of house dust endotoxin; medium or low levels of endotoxin had no significant effect. This result was independent of animal exposure. Total IgE was significantly lower in the CC allele exposed to stable animals with the reverse happening in exposure to dog/cat only. Similarly specific IgE was significantly higher

in dog/cat exposure with a trend (p = 0.057) for lower specific IgE in C allele exposure to stables. These results suggest that not only is dose concentration important but also that the particular type of animal exposure also plays a role.

The threshold effect of exposure to animals and presumably microbials has been replicated in epidemiological studies. Holbreich et al[108] found that atopic sensitisation and asthma prevalence was significantly lower in farming children compared to non farming children (atopy 25.2% and 44.2% and asthma 6.8% vs 11.3% respectively), but also found that other factors also play a role. They found that large family size seemed to confer additional protection with Amish farmers children (average offspring 5.9) having a lower prevalence of asthma of 5.2% and of atopy 7.2%). Timing of exposure to endotoxins and other microbial substances is important, exposure of children under 1 year to stables, and consumption of unpasteurised farm milk was associated with significantly lower prevalence's of asthma and atopic sensitisation compared to children aged 1 to 5 years[109].

In summary, timing, level of exposure, genetic factors as well as particular animal exposure all interact with the impact of endotoxins and other microbial substances on the risk of asthma and atopy.

1.12.3 Breast-feeding and Asthma

Some observational studies have confirmed a link between breast-feeding and reduction in allergic disease and asthma specifically[110,111]. Equally other observational studies have not shown a significant protective effect[112]. To further complicate matters whilst exclusive breast feeding until 4 months was associated with a significant reduction in asthma prevalence until 8 years of age[113], a prospective study from birth to 44 years confirmed the protective effect

of breast feeding for the first 7 years of life but also found that this effect was reversed in later life[114]. Interactions between breast feeding and parental atopy add to the complexity. In the Tucson birth cohort, breast-feeding was associated with an increased risk of asthma amongst atopic children with a family history of maternal asthma [115]. Breast-feeding duration seems to be an important factor in terms of lung function with longer duration of breast-feeding associated with better lung function profile in children followed from birth to 10 years[116].

1.12.4 Formula feeds and risk of allergic disease

Hypoallergenic formula feeds are processed by enzymatic hydrolysis. Various different protein sources are used, for example casein, whey, soya or amino acid mixtures. Products are further classified by the degree of hydrolysis they have undergone into extensively and partially hydrolysed protein products. Whilst there is no evidence that hydrolysed formula are more effective than breast milk at preventing asthma, and other allergic diseases there is evidence that in high risk infants who are unable to breast feed that these formula are more effective than cows milk in reducing the risk of allergic disease[117]. Extensively hydrolysed formula appears to be somewhat more effective than partially hydrolysed on reducing the risk of allergic disease[118]. In summary, feeding high-risk infants, who are not breast fed, hypoallergenic formula combined with avoidance of solid foods during the first 4-6 months reduces the cumulative incidence of cow's milk protein allergy and atopic dermatitis as compared with a standard cow's-milk-based formula[119].

1.12.5 Dietary factors

A number of studies show an association between specific foods and food groups and asthma. Up until 24 months one prospective study from Japan found that high intake of dairy products was protective against wheeze in infancy[120]. In contrast another study found that a diet with a low to moderate intake of dairy products and egg, and a low intake of red meat during pregnancy was associated with a reduced risk of childhood persistent wheeze, atopic wheeze and atopy at 6.5 years[121]. This is complimented by a study finding that sensitization to eggs and cows milk in the first year of life were independently associated with an increased risk of adult asthma[122]. Bread and butter consumption was significantly associated with wheeze and shortness of breath in children aged 7[118]. Nuts, particularly peanuts are associated with a higher risk of asthma in childhood, via maternal diet and also by early introduction of nuts in children's diet[123]. A further study found that eating fish more than twice a week by the mother during pregnancy was associated with reduced wheeze at 6 years[124].

The evidence supporting a preventive effect of exclusion diets during pregnancy is poor. A recent Cochrane review did not find evidence that maternal exclusion diets during pregnancy had a protective effect against asthma or atopic disease[117].

1.12.6 House dust mites

House dust mite is the commonest allergen to be sensitised to in UK populations[26]. Research has focused on HDM as a potent risk factor for the development of asthma with early studies suggesting a significant association between HDM sensitisation and asthma[125], especially in coastal regions [126]. Studies investigating the effects of inhalation of HDM allergens and bronchospasm in participants sensitised to HDM suggest that this is a causal association with inhalation of HDM allergen associated with increased hyperresponsiveness[127]

More recent research suggests that the issue of house dust mite avoidance may be more complex, the concentrations of HDM allergen exposure seem to be important in determining the risk of HDM exposure for asthma with Juan et al[128] finding that it is high concentrations of HDM allergens that are associated with increased prevalence of asthma at 7 years of age. Further evidence for varying concentrations of HDM allergen being a factor in risk of asthma comes from Tovey et al[129] who found that both low and high exposure to HDM over time were associated with reduced atopy and asthma compared to intermediate levels of HDM.

Secondary prevention

Given the multitude of studies showing a high prevalence of HDM sensitisation, an association between sensitisation and asthma, and studies showing a worsening of airflow obstruction on inhalation of HDM allergen avoidance of HDM exposure would be expected to reduce asthma symptoms. In adults however well constructed randomised control trials have not shown that lowering HDM levels leads to significant improvements in asthma symptoms or control[130]. In childhood some studies suggest an improvement in asthma control and

symptoms with HDM avoidance[131]. A Cochrane review assessing the efficacy of HDM avoidance in those with established asthma (secondary prevention) found no significant benefit of avoidance measures in terms of reduced symptoms or improvement in lung function[132].

Primary prevention

With regards to primary prevention, studies solely focusing on HDM avoidance have not shown a significant reduction in asthma, rhinitis, eczema or even sustained reduction in HDM sensitization[133], **Table 1.3**.

1.12.7 Family history

A family history of asthma is a significant risk factor for asthma onset with studies finding odds ratios for a first-degree relative of 1.5 to 9.7[134]. Maternal asthma appears to be more of a risk factor than paternal asthma with an odds ratio of 3.36 versus 2.67[135]. In a review of 14 studies Burke et al[134] found that a positive family history of atopy significantly increased the risk of asthma onset with odds ratios of 1.7 to 6.8. A combination of a familial history of atopy and asthma appear to be additive in increasing the risk of asthma onset[136], with a doubling of asthma risk associated with dual heredity of allergic disease[137]. Elevated cord IgE is associated with an increased risk of atopy[137,138], and hence, indirectly is a marker of increased risk of allergic disease.

1.12.8 Passive smoking

Children exposed to tobacco smoke in infancy are at increased risk of wheeze, asthma and respiratory tract infections[139,140]. The link between atopy and passive smoking is less clear cut, some studies have found exposure to smoking in infancy is associated with an increased risk of atopy[141] other studies have failed to find an association[142]. In terms of other allergic disease there is evidence of an association between eczema in childhood and maternal smoking[143] and rhinitis and exposure to tobacco smoke in the first year of life[144].

1.12.9 Male gender

A number of studies have consistently shown significant gender differences in the risk of atopy, asthma, wheeze and allergic disease. In childhood male gender significantly increases the risk of atopy[145,146], wheeze[147], asthma[148], rhinitis and atopic eczema[149]. Over adolescence this gender difference reverses with an increased prevalence in females[150].

1.13 Primary prevention of allergic disease

The goal of primary prevention is to prevent the onset of disease and can be broadly divided into two approaches:

1) Avoidance of risk factors and inhibiting their mechanisms of action.

For example, avoidance of aeroallergens and dietary allergens.

2) Promotion of protective factors and stimulating their mechanisms of action.

Allergen immunotherapy, Dietary supplements (antioxidants, omega-3 fatty acid etc.)

This is in contrast to secondary prevention where the aim is to reduce disease burden and/or progression.

1.13.1 Identifying suitable candidates for primary prevention

Targeting individuals who are more likely to develop asthma and allergic disease is obviously the ideal. Given that an intervention needs to occur as early as possible to avoid the onset of immunological and structural changes in the airway this does present a challenge. We know that atopy is a significant risk factor for asthma onset and has a strong heritable component. Therefore selecting candidates who are at high risk of developing atopy/allergic disease due to a family history of asthma/atopic disease in first-degree relatives will identify those individuals who are more at risk of asthma than the general population. This is partly because they are more at risk of developing atopy, which increases their risk of asthma but also because they are more likely to inherit genes that will predispose them to asthma directly if their relative has atopic asthma.

In terms of timing, we know that the majority of asthma begins in childhood and that changes consistent with airways remodelling are seen after the age of one, therefore any intervention needs to occur within the first year of life.

1.13.2 Strategies of primary prevention: a brief review of primary prevention studies

A number of studies have been designed to assess the effectiveness of manipulating environmental risk factors to reduce the risk of onset of asthma in individuals considered at high risk of asthma onset. Selection criteria have all been based on a family history of either asthma or atopic disease or both. Interventions have varied widely between studies, but broadly they can be divided into:

1. Single intervention studies where one aspect of environmental risk has been excluded – either house dust mite or dietary factors.

2. Multifaceted prevention where both house dust mite avoidance and dietary factors have been excluded.

1.13.3 Single Intervention studies

Single intervention studies have selected participants based on a history of maternal allergic disease, family history of asthma, family history of allergic disease in one or more first degree relatives, or dual parental atopy (Table 3). Interventions have either been based on avoiding house dust mite exposure or by modifying diet. Dietary modification has either been both mother (if breast feeding) and child or focused on the child avoiding allergens in the diet in the first year of life. No single intervention study to date has achieved a significant reduction in asthma (**Table 1.3**).

Table 1.3. Single intervention primary prevention studies

Study	Selection Criteria	Intervention	Age at last follow-up	Significant reduction in asthma achieved
Zeiger[151] n = 351	At least one parent with an atopic disease	Maternal diet. Caesin hydrolysate formula/breast feeding until 12 months	7	No
Mallet[152] n = 177	FHx atopic disease in first degree relatives	Caesin hydrolysate only for 4/12 in addition to or as a substitute for breast feeding	4	No
PIAMA[153] n= 811	Maternal allergic disease	HDM reduction*	4	No
SPACE[154] n = 696	Fhx atopic disease parents/siblings	HDM reduction*	2	No
CAPS[155] n = 616	At least 1 parent with asthma	HDM reduction*✶	5	No
MAAS[156] n=291	Both parents atopic	HDM reduction*✶	3	No

*Mattress and pillow covers infants and parents. ✶Acaricidal agent

1.13.4 Multifaceted primary prevention studies

Similarly to single intervention studies, multifaceted studies have varied in their selection of participants (**Table 1.4**). All interventions have combined house dust mite avoidance with dietary modification. All studies aside from the CAP study encouraged breast-feeding until at least 4 months, and where breast-feeding was not possible, used a hydrolysed substitute. The PREVASC study found significantly less asthma symptoms reported by the parents at two-year review and a non-significant trend for less GP attendances with asthma related symptoms in the intervention group[157]. At its most recent follow-up at 6 years this significance was lost. The CAPPS study found that at 7 and the Isle of Wight study at 8, that there was significantly less asthma in the intervention group compared to the control group.

The CAP study did not find a significant reduction in asthma up to and including at the 11.5-year review.

Table 1.4. Multifaceted intervention studies

Study	No	Selection criteria	Intervention	Age at last follow-up	Significant reduction in asthma achieved
The Canadian asthma primary prevention study (CAPPS)[158]	545	1 first degree relative with asthma or 2 first degree relatives with atopic disease	HDM reduction**Pets, ETS reduction Breast feeding 4/12 or partially hydrolysed formula	7	Yes
The Childhood Asthma Prevention study (CAPS)[159]	616	At least1 parent/sibling with asthma/frequent wheeze. Excluded pet cat in the home	HDM reduction** Diet fatty acid modification	11.5	No
Isle of Wight primary prevention of asthma	120	2 first degree relatives allergic disease or 1 plus high cord IgE	HDM** Diet Breast feeding/ extensively hydrolysed formula	8	Yes
The prevention of asthma in children study PREVASC[160]	443	1 first degree relative with asthma	HDM reduction*, Pet avoidance. Breast feeding/ 6/12 or hypoallergenic formula Reduction in ETS exposure pre and post natally	6	No

*Mattress and pillow covers infants and parents. *Acaricidal agent

1.13.5. Conclusions

Research over the last 40 years highlights the complexity of asthma, and that there are multiple phenotypes of asthma, as well as complex interactions between genetic predisposition to asthma, atopy, bronchial hyper-responsiveness and environmental factors.

Observational studies have demonstrated that both timing of exposure as well as dosage of environmental factors influence whether individuals will develop asthma, atopy and other allergic diseases. This implies that manipulating environmental factors may enable us to prevent the onset of allergic disease in those genetically predisposed.

We know that atopy is a significant risk factor for asthma onset and has a strong heritable component. Therefore selecting candidates who are at high risk of developing atopy/allergic disease due to a family history of asthma/atopic disease in first-degree relatives will identify those individuals who are more at risk of asthma than the general population.

In terms of timing, we know that the majority of asthma begins in childhood and that changes consistent with airways remodelling are seen after the age of one, therefore any intervention needs to occur within the first year of life.

Whilst studies focusing on single interventions have not been successful in reducing asthma [161] those focusing on multifaceted environmental manipulation have been more successful (Table 1.4). The Isle of Wight primary prevention of asthma study is one such multi-faceted intervention study, which at previous follow-ups has shown significant differences in the intervention group in terms of atopy, and allergic

disease compared to the control group. We will now report findings from the 18th year of follow-up.

1.14 The Isle of Wight Primary Prevention of asthma study

The Isle of Wight prevention study is the oldest established dual primary prevention study that is still active.

The original hypothesis behind the cohort study was that in high-risk individuals the prevalence of asthma and potentially other allergic diseases, could be significantly reduced by allergen avoidance instituted in the first 12 months of postnatal life[162].

1.14.1 Selection Criteria

Between March 1990 and February 1991 the study was discussed with 1116 pregnant women on the Isle of Wight, all 504 pregnant women who met the inclusion criteria of dual hereditary (allergic disease in both parents, one parent and one sibling, or two siblings) or single heredity (allergic disease in one parent or sibling) plus cord blood IgE > 0.5kU/l were asked whether they would participate in this study, 301 pregnant women agreed to participate.

Cord blood IgE was considered to be a potential marker of increased risk of atopy and allergy[163],[164]. Prior to the availability of total cord blood IgE, all mothers within the intervention group who were breast-feeding, followed dietary exclusion from the birth of their infant, until

the IgE results were available. 162 of the 194 participants with single heredity were excluded, as their cord blood IgE was less than 0.5kU/l.

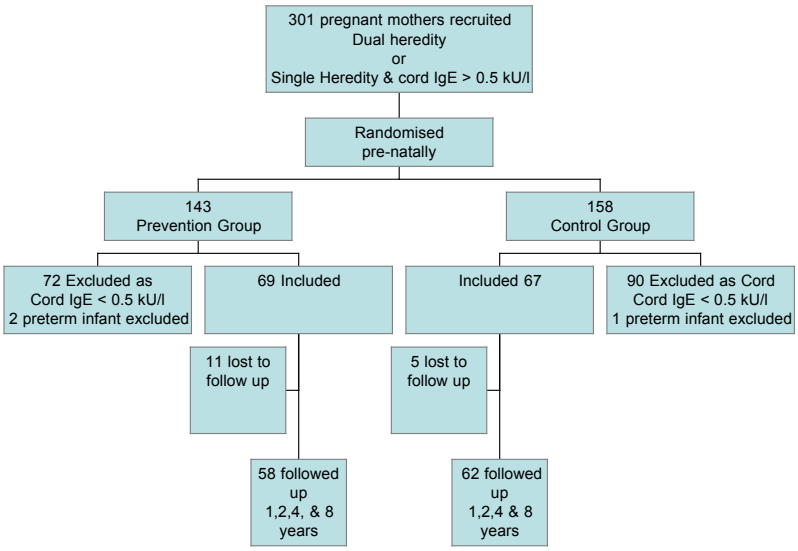
1.14.2 Intervention measures

Mothers who were breastfeeding were asked to follow a strict dietary regime excluding the following food groups: dairy products, egg, fish, soya and nuts. For up to 9 months breast feeds were supplemented when required, with an extensively hydrolysed soya formula. Children who were not breastfed received this supplement exclusively from birth. Dietary compliance was assessed by testing random samples of breast milk for cow's milk protein (casein and β-lactoglobulin).

Infant's diets excluded dairy products, egg, wheat, (unhydrolysed) soya products, oranges, fish and nuts until they were 9 months old. From 9 months of age cow's milk and soya were introduced, from 10 months wheat, and from 11 months eggs, at 12 months all dietary restrictions were lifted from the infant's diet.

All infants in the prevention group used polyvinyl covered mattresses (with a vented head area). In addition anti-dust mite treatment with acarosan foam and powder was applied to the infant's bedroom carpet, living room carpet, and upholstered furniture at birth, and this was repeated every 3 months until the infants were 9 months old. In the control group dust mite samples were collected at birth and at 9 months of age (ALK filter connected to a Hoover Dustette vacuum cleaner, 500 W). Baseline levels were collected from all participants within one week of discharge from hospital. The dust samples were assayed for mite antigen (Der p1) per unit weight (μg/g) using ELISA. Data relating to HDM levels was double entered into SPSS for analysis. The mean of the baseline visit and the visit of 9 months was used in analysis. Testing demonstrated a fivefold reduction in dust mite antigen in the prevention households compared to the control group[165].

Figure 1.5. Overview of recruitment and follow-up until 8 years



1.15 Previous follow-ups of the prevention cohort

The 12 month follow up consisted of physical examination by a paediatric allergy specialist, who was blinded to which group the infants were in, and assessment of the presence of asthma, eczema, and food intolerances. Skin prick testing was performed against house dust mite, grass pollen, cat dander, cow's milk, egg and any other allergen implicated in a particular case. Information on the presence of pets, parental smoking, and demographic data such as the social economic status, and whether the infant shared a bedroom with either their siblings or their parents.

Follow up at 24 months, and 4 years followed a similar pattern.

At 8 years children also underwent pulmonary function testing, methacholine challenge, exercise testing, and blood was taken to assess total IgE levels. Follow up to 8 years demonstrated a significant reduction in allergic disease in the prevention group compared to the control group[165].

1.16 Study hypotheses, aims and objectives at the 18-year follow-up

Environmental manipulation through the dual intervention of house dust mite avoidance, and dietary modification in participants at high risk of asthma will reduce the prevalence of asthma and associated allergic disease at 18 years by attenuating the influence of atopy on asthma onset.

This study aims to assess the effects of house dust-mite avoidance and dietary modification in the first year of life on the prevalence of asthma and associated allergic disease at 17 years post intervention

The objectives are to assess all 120 participants of the original study, using questionnaires, skin prick testing, spirometry, methacholine challenge, exhaled nitric oxide measurement and additionally, sputum induction in order to enable assessment of airway inflammatory cells.

Chapter 2: Methods: The Isle of Wight Primary Prevention of asthma and associated allergic disease: 18-year follow-up

2.1 Design of the study

This study is a randomised control trial testing the hypothesis that environmental manipulation through the dual intervention of house dust mite avoidance and dietary modification in high-risk participants will reduce the prevalence of asthma and allergic disease at 18 years.

2.2 Recruitment at 18-year follow-up

All 120 children originally recruited were approached in their 18th year. This was initially via an introductory letter containing information about the study and what participation would entail (appendix 1). Included with the letter was a reply slip, which consisted of consent to partake in the study and information regarding the availability of the participant. Where no reply slip was returned after two weeks from the date of the initial letter a second letter was sent to the prospective participant.

Where participants had moved, the National Strategic Tracing Service (NSTS) was used to locate participants. There were no exclusion criteria as this was a follow-up study of the original participants.

2.3 Study personnel

A multidisciplinary team based at the David Hide Asthma and Allergy research centre, St Mary's hospital Newport on the Isle of Wight conducted the 18-year follow-up.

The research fellow (MS) and the research nurse (BC) were the primary assessors of the participants and were blinded to the original grouping of the participants. LT provided support in an administrative role, and SM helped locate and contact the participants. MS was responsible under the supervision of HA and GR for the co-ordination of the study, planning of recruitment, physical examination of the participants and writing to the general practitioners with the results, as well as data entry and analysis.

2.4 Organisation of the study

The original participants were contacted, those who were able to attend the David Hide Centre were given an appointment and underwent assessment with questionnaire, skin prick testing, exhaled nitric oxide measurement, spirometry and methacholine challenge. If for any reason participants were unable to complete part of the assessment (e.g. due to respiratory infections), a further visit was scheduled. After completion of the initial assessment participants were re contacted and asked to undergo sputum induction – if they agreed a further appointment was made. For those unable to visit the David Hide centre, the research fellow (M.S) visited them, where possible, and undertook all assessments except for the methacholine challenge, FeNO and sputum induction. For participants who could not attend the centre and it was

impossible for the research fellow to visit them at home, a telephone questionnaire was completed.

2.5 Assessment at 18-year follow-up

2.5.1 Questionnaires

The following six self-administered questionnaires were used in this study. All participants completed the ISAAC written questionnaire, the additional 17/18-year questionnaire, and the pubertal developmental scale. The other questionnaires were used according to the presence of allergic disease, asthma and eczema. The questionnaires are to be found in appendix 2.

2.5.1.1 ISAAC written questionnaire

ISAAC was established in order to provide an internationally standardised research tool in order to examine the epidemiology of atopy and asthma[166]. The ISAAC written questionnaire was used to assess respiratory, nasal, and dermatological symptoms; as well as recording demographic information such as education, smoking status, birth order, and socio-economic status. This questionnaire was also used at the 8-year follow up.

2.5.1.2 Additional 17/18-year questionnaire

This questionnaire recorded smoking status, alcohol consumption, and compliance with asthma medication.

2.5.1.3 Pubertal Development Scale (PDS)

The self-reported PDS has been validated in epidemiological studies[167]. Given the age range of our participants we modified this scale to ask the year of onset for each item.

2.5.1.4 Symptom questionnaire

Only patients with allergic symptoms since the age of 8 years were asked to fill in this questionnaire. This questionnaire has been used since the first follow up at one year of age. We used this questionnaire to detect both the presence of allergic disease in the study and control group, as well as morbidity in terms of symptoms, level of medication use and the number of exacerbations.

2.5.1.5 Juniper's asthma specific quality of life questionnaire: AQLQ-S >12

All participants with asthma were asked to complete the AQLQ-S >12. The AQLQ-S>12 is a standardised asthma health questionnaire, which is suitable for use in participants over the age of 12 years of age[168]. The questionnaire examines different domains of asthma quality of life, symptoms, activities, emotional well-being and environmental factors on a scale of 1 to 7 (1 = severely impaired – 7 = not at all impaired), The higher the score the less severe the impairment.

2.5.1.6 SCORAD (Severity scoring of atopic dermatitis)

SCORAD is a validated questionnaire used to determine the severity of eczema[169],[170] and was used to characterise the severity of eczema in participants with atopic dermatitis.

2.5.2 Skin prick testing at 18-year follow-up

Skin prick testing (SPT) is a relatively non-invasive method of assessing the presence of allergen specific IgE antibodies in an individual. A positive skin prick demonstrates a Type I sensitivity reaction (IgE mediated) and is used as a marker of atopy. The protocol followed a standard technique[171] of a positive control (histamine dihydrochloride, 10mg/ml) negative control (physiological saline). A panel of 13 common inhalant and food allergens were tested (Alk Abello, Horsholm, Denmark), these consisted of: House dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*, cow's milk, hen's egg, soya, cod, wheat and peanut. Participants were asked prior to SPT to avoid antihistamines for at least 72 hours. The allergens tested were applied to the volar aspect of the forearm. The skin was then pricked using a sterile lancet following a standardised validated technique[171]. A skin allergen reaction was considered positive when the mean wheal diameter was at least 3mm greater than the negative control after 15 minutes. All positive results were recorded on a standardised sheet (appendix A). Atopy was defined by at least one positive reaction to the panel tested.

Skin prick testing was performed by MS, and BC with both researchers blinded to the participants group. To maintain consistency of results RL, who was also blinded to the participants grouping, performed all measurements of the wheal diameter. The measurements were largest diameter of the wheal (internal) and at 90° to the first mark and the final measurement being an average of the two (mm).

2.5.3 Pulmonary function tests: spirometry

The American Thoracic Society/ European Respiratory Society guidelines[172] were followed to ensure the validity and reproducibility of spirometry. Percentage predicted for height, age, weight and ethnic origin were derived and recorded.

The following measures were taken using the Koko system (nSpire Health, Longmont, USA):

FEV_1 (forced expiratory volume at one second): as recommended we used the highest of three FEV_1 measurements that were within 5% of each other).

FVC (forced vital capacity).

FEV_1/FVC.

PEF (Peak expiratory flow reading)

For consistency all subjects performed spirometry whilst seated. In participants who were unable, or refused, to undergo a bronchial challenge reversibility was assessed by repeating spirometry 10 minutes after 600 mcg of salbutamol was inhaled, via a large volume spacer. Reversibility was defined as a 12% increase in FEV_1.

2.5.4 Assessing airway hyper-responsiveness - methacholine challenge:

Methacholine challenge testing is a method of assessing airway responsiveness in an objective, and reproducible fashion. We followed the American Thoracic Society guidelines[58] for methacholine challenge. The protocols for the methacholine challenge is provided in appendix 3.

2.5.4.1 Inclusion and exclusion criteria for a methacholine challenge

All participants in the original study where asked to undergo a methacholine challenge. Any individual who did not wish to partake was excluded. Participants were excluded if they had had a lower respiratory tract infection (LRTI) and/or required oral corticosteroids in the preceding two weeks, and had taken short acting β_2 agonist within six hours, and long acting β_2 agonist within 12 hours, or had not abstained from caffeine intake for at least 4 hours prior to the test. Where possible the methacholine testing was rescheduled.

Any individual whose FEV_1 was less than 70% predicted for their height and age on the day of testing were excluded from the methacholine challenge, and underwent reversibility testing instead. In addition where the research fellow believed that the risk to the participant of morbidity was unacceptably high, the methacholine challenge was deferred.

For the female participants we did not perform a methacholine challenge unless they had a negative pregnancy test, or were menstruating at the time of the challenge.

2.5.4.2 Preparation

Methacholine (16mg/ml) sterile inhalation solution (Stockport pharmaceuticals, Stockport, U.K) was prepared by following a set protocol, the same individual prepared the methacholine solutions for each challenge (MS), and the preparation was witnessed by one of the following researchers (BC, SM, AR). Each batch consisted of nine concentrations of methacholine doubling in concentration each time from 0.0625 mg/ml to 16 mg/ml and a control of 0.9% sterile saline. The batches were prepared on the day of the test and were stored in a fridge at 4^{o}C until an hour before the test.

2.5.4.3 Method

An experienced research registrar (MS) and research nurse performed the methacholine challenge. A 5-breath dosimeter method was used with a computerised dosimeter system (Koko Digidoser, PDS nSpire, Longmont, USA), with a nebuliser output of 0.01 ml/breath (KoKo DigiDoser Clinical Feature Sheet).

A pre test spirometry reading was performed to exclude participants with a FEV_1 less than 70% predicted. A 'control' solution of 0.9% saline was administered by 5 actuated inhalations of the saline followed by spirometry one minute later to obtain the base value of their FEV_1. For quality control three FEV_1 readings within 5% of each other were recorded, and the highest FEV_1 was used as the baseline.

In any individual with a 10% drop in their FEV_1, the test was postponed for an hour, if their FEV_1 dropped by 15% the test was cancelled and the individual underwent reversibility testing instead.

Stages 1-9

Methacholine dilutions were administered on continuous 5 minute cycles. At each stage participants inhaled 5 breaths of the solution, and 1minute later FEV_1 values were obtained. For quality control three FEV_1 readings within 5% of each other were recorded. Providing participants FEV_1 did not drop by more than 20%, increasing concentrations of methacholine were administered starting at 0.062mg/ml and increasing to 16mg/ml. The challenge continued until either the maximum dose of methacholine was given or there was 20% or more drop in the FEV_1. At the end of the study subjects were given a bronchodilator (e.g. 600mcg salbutamol via large volume spacer) and observed until their FEV_1 had returned to their baseline level. The results were recorded on a computerised sheet (Appendix A). Bronchial responsiveness was defined in terms of its relationship to the PC_{20}.

2.5.4.4 Calculating the PC_{20}

The PC_{20} is that concentration of methacholine, which causes a 20% drop in FEV_1. It is calculated using the following equation:

$$PC_{20} = -\text{antilog}\ [(\log C_2 - \log C_1)(20 - R_1) / \log C_1 + R_2 - R_1]$$

Where:

C_1 = second to last methacholine concentration

C_2 = Final methacholine concentration (the concentration resulting in a 20% or greater fall in the FEV_1

R_1 = percentage fall in FEV after C_1

R_2 = percent fall in FEV_1 after C_2

See Table 2.1 for a worked example.

Table 2.1. Calculating the PC_{20}

Post saline FEV_1 (mL)	2^{nd} last FEV_1 (mL)	Last FEV_1 (mL)	Concentration at 2^{nd} last FEV_1 (mg/ml)	Concentration at last FEV_1 (mg/ml)
3190	2930	2520	4	8

1^{st} % Fall	2^{nd} % Fall	PC_{20} (mg/mL)
8.15	21.00	7.58

Table.2.2.ATS definitions

Bronchial responsiveness	PC_{20}
Normal	>16mg/ml
Borderline	4-16mg/ml
Mild/positive	1-4mg/ml
Moderate-severe	< 1 mg/ml

2.5.4.5 Calculating the dose response slope

In order to be able to compare bronchial hyper-responsiveness where a participant's PC20 could not be calculated, as they did not have a 20% (or more) drop in their FEV_1 the dose response slope was calculated. This provided a continuous variable for all participants who successfully completed a methacholine challenge.

The dose response slope represents the % decline in FEV_1 from the post-saline value to the final dose of methacholine that was administered divided by the cumulative dose of methacholine administered[173].

Calculation of the dose response slope is therefore

Dose response slope = % decline in FEV_1 from the post-saline FEV_1 / by the cumulative dose of methacholine used. Where % decline FEV1 = (FEV_1 saline – FEV_1 final methacholine stage)/FEV_1 saline) X 100

Cumulative dose is shown in **Table 2.3.**

Table.2.3. Cumulative doses for calculating the DRS

Methacholine stage	Number of breaths	Concentration per stage (mg/ml)	Nebuliser output 0.01 ml/per breath	*Dosage per stage	Total cumulative dose (mg/ml)
1	5	0.06	0.01	0.003	0.003
2	5	0.12	0.01	0.006	0.009
3	5	0.25	0.01	0.0125	0.0215
4	5	0.5	0.01	0.025	0.0465
5	5	1	0.01	0.05	0.0965
6	5	2	0.01	0.1	0.1965
7	5	4	0.01	0.2	0.3965
8	5	8	0.01	0.4	0.7965
9	5	16	0.01	0.8	1.5965

Dosage per stage = 0.01 x 5 x Concentration per stage

Methacholine challenge at 8-year follow up

Results from methacholine challenge at 8 were re-analysed using the above method to ensure consistency in the comparisons between 8 and 18- year data.

2.5.5 Markers of airways inflammation

2.5.5.1 Exhaled Nitric oxide (FeNO)

Exhaled nitric oxide (FeNO) correlates highly with eosinophilic airway inflammation.[174]·[175] Studies comparing FeNO to induced sputum[176]·[177], bronchoalveolar lavage,[178] and bronchial biopsies[179]·[180], demonstrate significant correlations between levels of FeNO and the presence of eosinophilic airway inflammation. FeNO therefore serves as a useful marker of eosinophilic airway inflammation, especially with regards to asthma associated with atopy[181].

All participants in this study had their FeNO measured (Niox mino,® Aerocrine AB, Solna, Sweden) in accordance with the American Thoracic Society guidelines[182]. All patients were asked to refrain from eating, drinking, using beta-agonists, and were checked prior to measurement to ensure that they had not had a recent LRTI and/or had had oral steroids in the previous 14 days. We did not specify a standard time for measurement of FeNO, as there is no evidence of significant diurnal variation[183].

A biofeedback mechanism was used to maintain the expiratory flow rate at 50 ml/s and subjects exhaled against resistance to prevent upper airway contamination. Measurements were made in a standardised manner with the subject standing without a nose clip. All measurements were undertaken before spirometric testing, bronchial challenge and/or sputum induction.

2.5.5.2 Airway inflammatory cells from induced sputum

Sputum induction is a non-invasive method of obtaining airway secretions for the analysis of their cellular and biochemical constituents. Cellular analysis enables phenotyping of asthma, with elevated eosinophils found in asthma associated with atopy. In contrast, non-atopic asthma is characteristically associated with elevated neutrophils in induced sputum. European Respiratory Society (ERS) guidelines were followed in inducing and processing sputum[184]. Methods of sputum induction, and processing used in this study are to be found in appendix 4.

2.5.5.2.1 Sputum induction

Participants were excluded from sputum induction if they had had a lower respiratory tract infection and/or required oral steroids in the preceding 14 days.

Two protocols were used in inducing sputum.

1) The standard protocol for individuals whose spirometry was normal, or if abnormal their FEV1 was >60% predicted, and/or had airway hyper responsiveness, which at worst on methacholine testing was mild/positive (PC_{20}>1mg).

2) Participants who demonstrated moderate to severe airway hyper-responsiveness on methacholine testing and/or had an FEV_1 < 60% predicted underwent a modified protocol, in addition where the researcher suspected that an individual was at risk of bronchial hyper-reactivity the modified protocol was used.

Sputum was induced using an ultrasonic nebulizer (Devilbliss), with 40 ml 4.5% saline. Participants had their spirometry checked (baseline) and then 400μg of salbutamol (four puffs) was administered via a spacer, their post bronchodilator spirometry was then measured after 10 minutes. Subjects inhaled the nebulized solution for 5 minute intervals for a maximum of 20 minutes. At the end of each 5 minute interval their spirometry was repeated and the participant was encouraged to expectorate into a Petri dish. The procedure was terminated if the FEV_1 dropped by more than 20% of the post bronchodilator value, or if the participant complained of troublesome symptoms. The sample was kept on ice during collection, and refrigerated until processing occurred. If a participant had an FEV_1 <60% predicted or had a PC_{20} < 1mg then a modified protocol was used. Here the nebulizer was initially filled with 0.9% sterile saline solution and induction was performed for 30 seconds, before measuring FEV_1 Providing FEV_1 did not drop by >20%, nebulization time was increased to 1 minute and then 5 minutes. Providing FEV_1 remained stable the nebulized solution was increased to 3% and the procedure was repeated as for stage 1. If this failed to induce sputum 4.5% saline was used to induce for 30 s and 1, 2, 4 and 8 minutes.

2.5.5.2.2 Sputum processing

Sputum was processed by MS within four hours of collection. The liquid component was extracted and frozen at -80°C. The cellular component was analysed by MS according to ERS protocol. Sputum plugs, and

obvious mucoid components were selected from the expectorate and weighed. 22.5 μl of protease inhibitor per gram of sputum and DTT was added at 4 times the weight of sputum. The sample was then placed on a cell rocker for 45 minutes before being sieved through a sterile nylon mesh. This was then centrifuged for 10 minutes (1500 rpm). The supernatant was then separated and frozen at -80°C. The cell pellet had 1 ml of phosphate buffer solution added. 10 μl was then removed and added to 90 μl of 0.1% trypan blue (Sigma Aldritch Company Ltd, Gillingham). A manual cell count was then performed using a haemocytometer to record the total cell count. An appropriate amount of phosphate buffer solution was added to the cell pellet to achieve a cell concentration 1 x 10^6 UNITS. 70 μl of the suspended cells were added to each of four cytospin holders using filter cards and microscope slides (Shandon) and centrifuged at 400 rpm for 5 minutes. The slides were air dried for a minimum of 24 hours. The slides were then stained using the Romanowsky method (Rapi Diff II stain pack), and left to air dry for a minimum of 24 hours before being counted. A differential cell count of 400 inflammatory cells (macrophages, neutrophils, eosinophils, epithelial cells and lymphocytes) was performed. In addition squamous epithelial cells were counted in order to estimate saliva contamination.

2.6 Data Management

1. Current name and address were stored in an Excel database. This was kept separate from the SPPS database.

2. An SPSS database, using only the study number for identification, was used to record all results. MS was the primary data enterer and LT the second.

3. Confidentiality of all samples, interviews, and medical records was maintained by a) keeping all records under lock and key, b) separating data from names, c) keeping the linkage study numbers

under lock and key, d) allowing only study staff members to have access to the data, e) keeping identifiers of individuals out of public material and reporting only aggregated data.

2.6.1 Data at 0, 1,2,4 and 8 year follow-ups

Data relating to house dust mite levels during the period of intervention were double entered into an SPSS database, and analysed for the participants seen at 18 years.

To ensure consistency of results across the whole period of follow up all data on skin prick testing from previous follow-ups were re-measured by RL and were then double entered into an SPSS data-base by MS and LT. Data relating to spirometry, and methacholine challenge from 10 years follow-up were re-analysed. Specifically the dose response slopes were re-calculated to ensure validity of comparisons of bronchial hyper-responsiveness.

2.7 Definitions used in the analysis

2.7.1 Atopy

A skin allergen reaction was considered positive when the mean wheal diameter was at least 3mm greater than the negative control after 15 minutes. Atopy was defined by at least one positive reaction to the panel tested.

2.7.2 Asthma

The final definition used for analysis was derived from the ISAAC core questionnaire as a positive response to

'Have you ever had asthma?' *And* 'was it physician diagnosed?'

And a positive response to:

'Have you had wheezing or whistling in the chest in the last 12 months?'

If a participant had no wheeze, but was on prophylactic inhaled corticosteroids with physician diagnosed asthma then they were defined as having current asthma.

Atopic asthma was diagnosed as the above in the presence of atopy.

2.7.3 Eczema

'Have you ever had an itchy rash which was coming and going for at least 6 months?' *and* 'Have you had this itchy rash at any time in the last 12 months?'

Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes?

2.7.4 Rhinitis

'Have you ever had hay fever?' *And/or* 'Have you ever had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu?'

In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu?

Rhinitis was defined as persistent if it was present for more than 4 days a week or for more than 4 weeks a year, or as intermittent if it occurred less than 4 days a week or less than 4 weeks a year. Rhinitis was defined as mild if participants reported no interference with any of the

following: Sleep, daily activities, work and or school, and moderate to severe if participants reported impairment with these activities.

2.8 The primary and secondary end points at 18 year follow-up

The primary end point is the difference in the prevalence of asthma between the intervention and the control group.

Secondary end points:

1) Asthma severity
   Measured by asthma quality of life questionnaires and the asthma medication requirements of the participants
2) Airway inflammation:
   As measured by levels of exhaled nitric oxide (ppb)

   As measured by induced sputum inflammatory cells.
3) Lung function assessed by spirometry
4) Bronchial hyper-responsiveness:
   Defined by the response to a methacholine challenge.
5) Atopy as measured by the number of positive skin prick tests
6) Eczema.
7) Rhinitis

2.9 Statistical Methods

2.9.1 Power analysis

The power of a statistical test is the probability that the test will reject the null hypothesis when the alternative hypothesis is true (i.e. will not make a type II error).

1) Hypothesis:
   The hypothesis is that dual intervention will reduce the prevalence of asthma in the prevention group. The null hypothesis is that there will be no difference

2) Variability of outcome measure:
   We expected 8 of 58 (13.8%) children in the prophylactic group and 20 of 62 (32.3%) in the control group to have physician-diagnosed asthma

With Alpha = 0.05 the power of the study to detect this expected outcome is 0.6366 ((Lenth, R. V. (2006-9). Java Applets for Power and Sample Size from http://www.stat.uiowa.edu/~rlenth/Power.). Given the design of the study, as a follow up to an earlier interventional study, we were unable to further increase the sample size.

2.9.2 Analysis of primary and secondary outcomes

SPSS version 18 (Chicago, USA) was used for analysis. Continuous variables with a normal distribution, or amenable to transformation to a normal distribution, were analysed using T tests. Where continuous variables were not normally distributed non-parametric methods were used – the Mann-Whitney U test being the primary analysis performed.

Regression models, General Linear models (ANOVA and ANCOVA) and linear models were used to assess and adjust for potentially confounding factors for both primary and secondary outcomes. Categorical data was analysed using the chi-squared test, with logistic regression models used for multivariate analysis.

2.9.3 Longitudinal analysis

A pertinent criticism of cross sectional analysis is that it only enables one to show the presence or absence of an association between an outcome and an exposure at one point in time. Looking specifically at asthma in childhood and adolescence a significant difficulty with cross sectional analysis is that asthma remits and relapses, therefore measurement of prevalence at one point in time may 'miss' those children with asthma that has remitted at that particular point in time. Cross sectional studies are therefore vulnerable to type II error – that is not finding a significant association when one does exist (failing to reject the null hypothesis). In contrast longitudinal analysis increases the likelihood of discovering a true exposure/outcome relationship[185] and are thus more likely to detect potentially causal relationships. Generalized estimating equations (GEE) are a method of analysing longitudinal data, especially binary and/or count data and adjust for correlation over time[186] because variables of the model at different time points are analysed simultaneously, this makes chance findings because of multiple testing less likely than when using logistic regression analyses for all the outcomes at different time points separately.

2.10 Ethical approval

2.10.1 NHS Research Ethics Committees (RECS)

The fundamental aim of RECs is firstly to protect research participants and secondly to promote ethical research. Review by an NHS REC is mandatory where the aim of the proposal is to generate or test hypothesis either quantitatively or qualitatively. There are different types of RECs across the UK, which review different types of studies. RECs recognised by the United Kingdom Ethics Committee Authority (UKECA) are able to review clinical trials of medicinal products (CTIMPs). Certain RECS are 'flagged' as having expertise in reviewing particular types of research.

Applications are now done online via the Integrated Research Application System (IRAS).

2.10.2 NHS management permission "R & D approval"

In addition to REC approval all research involving human participants carried out within NHS organisations requires that the following requirements have been met:

- There are adequate arrangements and resources to meet the standards set out in the Department of Health's Research Governance Framework for Health and Social Care (RGF)
- An identified sponsor has taken on responsibility for the study;
- The study has received ethical approval (where required);

- There is a clinical trial authorisation in place for a clinical trial of a medicine, (not applicable for this study);
- The allocation of responsibilities is agreed and documented;
- Appropriate contractual arrangements are in place;
- Legislation relating to the research is followed within the organisation;
- A person authorised to do so has given written permission on behalf of the NHS organisation.

The process of conducting the above checks and giving written permission is called NHS management permission for research, often described as R&D approval. In most NHS organisations an R&D office or network is responsible for carrying out the relevant checks.

2.10.3 Amendments and further applications

Amendments are changes made to the research after a favorable ethical opinion has been given. They can be 'substantial' or 'non-substantial'. A substantial amendment is defined as an amendment to the terms of the application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

1. The safety or physical or mental integrity of the subjects of the trial;
2. The scientific value of the trial;
3. The conduct or management of the trial; or
4. The quality or safety of any investigational medicinal product used in the trial.

Some changes, however, will have no significant implications for participants or for the conduct, management or scientific value of the study and can be regarded as 'non-substantial' or 'minor' amendments.

The chief investigator for the study is required to submit the NRES notice of substantial amendment form to the main REC only.

A Substantial amendment needs to include:

1. The change(s) included in the amendment and briefly explain the reasons in each case on the notice of amendment, using language comprehensible to a layperson.
2. The documents that have been modified, showing both the previous and new wording, with the form.
3. The sponsor or chief investigator may also include other supporting information, such as a summary of trial data, an updated safety analysis or a report from a trial monitoring committee.

4. Where the amendment could significantly affect the scientific value of the

Research, further evidence of scientific and/or statistical review is required.

2.11 Ethical approval for The Isle of Wight Primary Prevention of asthma and associated allergic disease: 18-year follow-up

Ethical approval was gained from the NHS REC prior to approaching potential participants (Southampton & South West Hampshire Research Ethics Committee, (07/HO504/188). Please see appendix 5

A notice of substantial amendment requesting a substitution of a quality of life asthma questionnaire for one that was validated in teenagers was accepted in July 2008

A notice of substantial amendment requesting the additional test of sputum induction was rejected; the ethics committee recommended this was submitted as a full research proposal.

A full research proposal generating a separate study based on sputum induction of the 120 participants was consequently submitted and accepted by the Southampton & South West Hampshire Research Ethics Committee November 2008. Please see appendix 6.

Since this study was completed the process for applying for ethics and R&D permission has been further simplified by the development of an Integrated Research Application system (IRAS). IRAS is a single system for applying for the permissions and approvals for research in the UK. Rather than multiple separate application forms the information about the research project is entered once. Within the application process are multiple filters ensuring that the data collected and collated is appropriate to the type of study and that the appropriate permissions and approvals are requested. For example IRAS captures the information required by the following review bodies:

- Administration of Radioactive Substances Advisory Committee (ARSAC)
- Gene Therapy Advisory Committee (GTAC)
- Medicines and Healthcare products Regulatory Agency (MHRA)
- NHS / HSC R&D offices
- NRES/ NHS / HSC Research Ethics Committees
- National Information Governance Board (NIGB)
- National Offender Management Service (NOMS)
- Social Care Research Ethics Committee

Chapter 3: Previous follow-up at 1, 2, 4 and 8 years

3.1 Introduction

The results from previous follow-up of the intervention were analysed with reference to the participants seen at 18, this was to ensure consistency of diagnosis at each time point in order to enable a meaningful longitudinal analysis to take place.

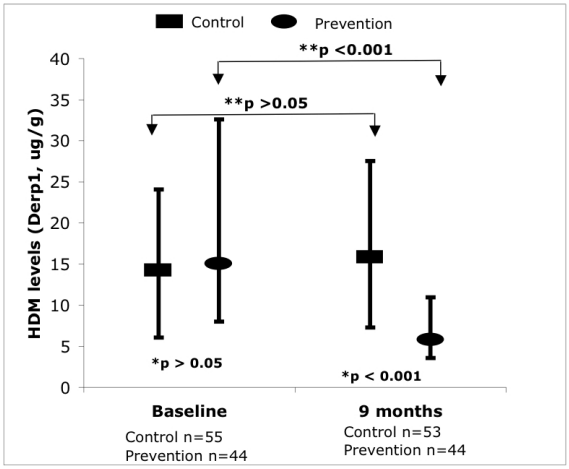
3.2 Analysis of house dust mite levels at 0 and 9 months

The initial collection of HDM samples (baseline) was taken in the first month of life, at this stage there were no significant differences between the control and prevention group (median 14.67 Der p1, μg/g (interquartile range 6.03-24.07) versus 15.04 Der p1, μg/g (7.98-32.59), p =0.336). The HDM samples taken at 9 months showed that prevention group had significantly lower levels of HDM than the control group (5.86 (3.55-10.93) versus 15.31 (7.25-27.49), p <0.0001). Comparing levels of HDM for the prevention at baseline and 9 months confirmed that they were significantly different (p< 0.0001) (**Figure 3.1**).

In our study at 18-year follow-up we used the median to compare the control and intervention groups before and after using acaricide in the intervention group, as the data was not normally distributed. Using the geometric mean, HDM levels at baseline for the prevention group were 1.23 Der p1, μg/g pre-intervention and 0.81 Der p1, μg/g post-intervention (9months), for the control group 1.15 Der p1, μg/g, pre-intervention and 1.15 Der p1, μg/g at 9 months.

Reviewing the literature on reported HDM levels in the UK our study compares favourably with the geometric mean reported in other studies Sporik et al reported the geometric mean as ranging from 2.4 Der p1, μg/g in the living room, 4.3 Der p1, μg/g in the bedroom floor and 18.4 Der p1, μg/g in the mattress in Poole, Dorset[187]. Custovic et al in Manchester reported the geometric mean Der p 1 concentrations in house dust samples from the homes of 53 subjects as 3.6, 1.2, and 4.4 μg/gm in mattresses, bedroom carpets, and bedding[188]. In Norwich geometric mean concentrations of Der p 1 were 1.9 μg/g in living room floor dust, 1.7 μg/g in bedroom floor dust and 2.0 μg/g in mattress dust[189].

Figure.3.1. Comparison of median House dust mite levels at birth and at 9 months



Results represent medians (markers) with lines representing the interquartile range for each.

*Mann-Whitney U Test, **Wilcoxon signed ranks test

3.3 Atopy and skin prick sensitisation (SPT)

Not all participants underwent skin prick testing at each follow-up. Additionally there were differences at each follow-up in terms of the specific allergens tested, and individual differences between participants as to whether they underwent the full panel of testing at each follow-up. Atopy at each follow-up represents overall prevalence of atopy at that time point with reference to the participants seen at 18-year follow-up. For this study at all follow-ups 3mm wheal size has been used as the cut off for a positive result to ensure consistency in analysis.

3.3.1 Atopy and SPT results at 1-year follow-up

At 1-year follow-up there were no significant differences between the groups in terms of sensitization to specific allergens, or the prevalence of atopy (**Table 3.1).**

Table 3.1. Skin prick sensitisation at 1 year follow up

Allergen	Prevention	Control	p value*
House dust mite	0/26 (0%)	0/39 (0%)	n/a
Cat	0/19 (0%)	1/32 (3.1%)	1**
Dog	0/13 (0%)	0/13 (0%)	n/a
Grass	0/20 (0%)	0/31 (0%)	n/a
Milk	0/25 (0%)	0/35 (0%)	n/a
Egg	2/25 (8%)	4/34 (11.8%)	1**
Wheat	0/20 (0%)	0/29 (0%)	n/a
Atopy at 1 year	2/30 (7%)	4/40 (10%)	0.694*

Numbers represent number of participants with sensitisation or atopy over total number of participants tested.

*Pearson Chi-square test unless otherwise stated.** Fishers exact test

3.3.2 Atopy and SPT results at 2-year follow-up

At the 2-year follow-up defining a positive skin prick result using a 3mm cut off the prevalence of atopy was not significantly different between the prevention and control groups, 2/53 versus 7/56, p = 0.162 respectively (**Table 3.2**.).

Table.3.2. Skin prick sensitisation at 2-year follow-up.

Allergen	Prevention	Control	p value*
House dust mite	0/55 (0%)	3/56 (5.4%)	0.243**
Cat	1/55 (1.8%)	4/56 (7.1%)	0.364**
Dog	1/55 (1.8%)	1/55 (1.8%)	1**
Grass	0/55 (0%)	0/56 (0%)	n/a
Cladosporuim	0/55 (0%)	0/55 (0%)	n/a
Alternaria	0/55 (0%)	0/55 (0%)	n/a
Milk	0/55 (0%)	0/55 (0%)	n/a
Egg	2/55 (3.6%)	2/55 (3.6%)	1**
Wheat	0/55 (0%)	0/55 (0%)	n/a
Soya	0/55 (0%)	0/55 (0%)	n/a
Atopy at 2	2/55 (4%)	7/56 (13%)	0.162*

Numbers represent number of participants with sensitisation or atopy over total number of participants tested.

*Pearson Chi-square test unless otherwise stated.** Fishers exact test

3.3.3 Atopy and SPT results at 4-year follow-up

At 4 years post intervention follow-up the prevalence of atopy was significantly lower in the prevention group compared to the control group: 11% versus 28%, p <0.05 (**Table 3.3).**

Table 3.3. Skin prick sensitisation at 4 year follow up.

Allergen	Prevention n (%)	Control n (%)	P value*
House dust mite	3/52 (5.5%)	9/56 (16%)	0.072**
Cat	1/55 (1.8%)	4/52 (7.1%)	0.364
Dog	1/54 (1.8%)	3/53 (5.4%)	0.618**
Grass	2/55 (3.6%)	4/55 (7.3%)	0.679**
Tree	0/53 (0%)	1/56 (1.8%)	1**
Cladosporuim	0/53 (0%)	2/56 (1.8%)	0.496**
Alternaria	1/53 (1.9%)	3/56 (5.4%)	0.619
Milk	0/55 (0%)	2/56 (3.6%)	0.495**
Egg	1/55 (1.8%)	1/56 (1.8%)	1**
Wheat	0/53 (0%)	1/56 (1.8%)	1**
Peanut	0/53 (0%)	0/53 (0%)	n/a
Cod	1/53 (1.9%)	0/56 (0%)	0.486 **
Soya	0/53 (0%)	0/53 (0%)	n/a
Atopy at 4	6/55 (11%)	16/57 (28%)	**0.022**

Numbers represent number of participants with sensitisation or atopy over total number of participants tested.

* Pearson Chi-Square test unless otherwise stated.** Fishers exact test

3.3.4 Atopy and SPT results at 8-year follow-up

At 8-year follow-up the prevalence of atopy was significantly lower in the prevention group compared to the control group (**Table 3.4**). The prevalence of house dust-mite sensitisation was also significantly lower in the prevention group versus the control group.

Table 3.4. Primary prevention, skin prick test results at 8 year follow up.

Positive results at 8 year follow-up	Prevention n (%)	Control n (%)	p value*
House dust mite	5/53 (9.4%)	18/58 (31.0%)	**0.005**
Cat	3/53 (5.7%)	7/58 (12.1%)	0.326**
Grass	8/53 (15.1%)	13/58 (22.4%)	0.325
Tree	0/53 (0%)	4/58 (6.9%)	0.120
Milk	0/53 (0%)	2/58 (3.4%)	0.496**
Egg	0/53 (0%)	0/58 (0%)	n/a
Peanut	0/53 (0%)	0/58 (0%)	n/a
Cod	0/53 (0%)	0/58 (0%)	n/a
Atopy at 8	9/45 (17.0%)	24/58 (41.0%)	**0.004**

Numbers represent number of participants with sensitisation or atopy over total number of participants tested.

* Pearson Chi-Square test unless otherwise stated.** Fishers exact test

3.4 Prevalence of asthma at previous follow-up

The prevalence of asthma for those participants reviewed at 18 was not significantly different between the 2 groups at any prior follow-up (**Table 3.5**).

Table 3.5. Prevalence of asthma at previous follow-up

Year of follow-up	Prevention n =56 (%)	Control n=58 (%)	*p value
1	4 (7.1)	11 (19.0)	0.062
2	9 (16.1)	15 (25.9)	0.200
4	14 (25.0)	20 (34.5)	0.269
8	10 (17.9)	15 (25.9)	0.302

Numbers represent number of participants (percentage).

* Pearson Chi-Square test.

3.5 Prevalence of eczema

At 1-year follow-up there was a significant difference in the prevalence of eczema, with the prevention having less participants with eczema than the control group. This trend of less eczema in the prevention group continued throughout previous follow-up but did not achieve statistical significance (Table 3.6.)

Table 3.6. Prevalence of eczema at previous follow-up

Year of follow-up	Prevention n =56 (%)	Control n=58 (%)	*p value
1	4 (7.1)	12 (20.7)	**0.037**
2	8 (14.3)	14 (24.1)	0.183
4	8 (14.3)	15 (25.9)	0.124
8	7 (12.5)	10 (17.2)	0.447

Numbers represent number of participants (percentage).

* Pearson Chi-Square test.

3.6 Prevalence of rhinitis

No attempt was made to diagnose rhinitis in the first year of life due to the obvious difficulty of diagnosis in this age group. There was a trend for less rhinitis to be present in the prevention group but this did not reach statistical significance at any stage of previous follow-up (**Table 3.7**).

Table 3.7. Prevalence of rhinitis at previous follow-up

Year of follow-up	Prevention n =56 (%)	Control n=58 (%)	*p value
1	n/a	n/a	
2	2 (3.6)	6 (10.3)	0.272**
4	6 (10.7)	11 (19.0)	0.216
8	16 (28.6)	21 (36.2)	0.384

Numbers represent number of participants (percentage).

* Pearson Chi-Square test. ** Fisher's Exact Test.

3.7. Summary:

A statistically significant reduction in HDM levels was achieved in the prevention group compared to the control group at 9 months (Figure 3.1). At 4 and 8-year follow-up there were significantly less participants who were atopic in the prevention group compared to the control group (Tables 3.3 & 3.4). There was a none significant trend for there to be less asthma in the prevention versus the control group from 1 to 8-year follow-up (**Table 3.5**).

Chapter 4: Cross-sectional analysis at 18-year follow-up

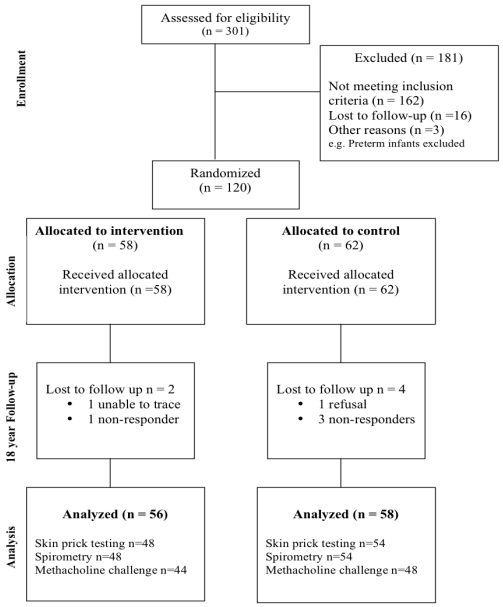
4.1 Introduction to the 18-year follow-up

There were 120 participants who took part in the randomised controlled intervention trial in 1990.They were therefore the participants who met the inclusion criteria for the 18-year follow up.

4.2 Flow of participants.

The progress of participants through the trial is shown in the CONSORT diagram (**Figure 4.1).**

Figure 4.1: Consort diagram showing flow of participants at 18-year follow-up



4.3 Recruitment at the 18-year follow-up

At the 18 year follow up, 119 were traced and contacted initially by letter, and then where possible by telephone. We received a response from 115, one declined to take part in the study. We received no response from 4 individuals. 114 (95%) of the original participants agreed to take part in the 18 year follow up. There were no significant difference in follow up between the two groups ($p > 0.05$, Pearson's Chi Square) with 96% (56/58) of the prevention and 93% (58/62) of the control groups agreeing to participate.

4.4 Mode of participation at 18-year follow-up

There was no significant difference between the two groups in their mode of participation, with the majority attending the research centre (**Table 4.1**.). One potential participant refused to participate in this study.

Table 4.1. Mode of participation in the 18-year follow-up

Method of participation	Prevention n=56	Control n = 59	*p value
Attended Centre	49 (88%)	51 (88%)	0.148
Home visit	0	3 (5%)	
Telephone questionnaire	7 (12%)	4 (7%)	

*Pearson's Chi-square Test

4.5 Characteristics of participants at 18-year follow-up

4.5.1 Demographics

There was no significant difference in gender, age, being in full time education, participants smoking, exposure to tobacco smoke in the home or cat exposure **(Table 4.2).**

Table 4.2. Characteristics of the participants at 18-year follow-up

Variable	Prevention (n=56/58)	Control (n=58/62)	p value*
Participated in the 18 year follow up	56 (96.6%)	58 (93.5%)	0.451
Age (years)	18.38 (0.41)	18.47 (0.40)	0.995**
Male gender	27 (48.2%)	34 (58.6%)	0.265
In full time education	37 (66.1%)	42 (72.4%)	0.463
Living with parents	48 (85.7%)	53 (91.4%)	0.341
Total annual family income	1-5[†]	1-5[†]	0.824
Current smoking	23 (41.1%)	19 (32.8%)	0.358
Exposure to smoking in the home in the last 2 years	17 (30.4%)	26 (44.8%)	0.111
Cat at home in the last 2 years	20 (35.7%)	21 (36.2%)	0.956

Numbers are frequencies (percentages) or means (SD)

* Pearson's Chi-square test, ** Independent samples T Test.

[†] 1= < £12,00, 2 = £12,000-17,999, 3 = £18,000-29,999, 4 = £30,000-41,999, 5= > £42,000

4.5.2 Heredity and early life factors

Significantly more participants were firstborn in the control group compared to the prevention group. In contrast there were more individuals who had 'dual heredity' (that is both parents having an 'allergic disease') in the prevention group. Other early life factors were not found to be significantly different **(Table 4.3.).**

Table 4.3. Comparison of heredity and early life risk factors at 18-years follow-up

Variable	Prevention (n=56)	Control (n=58)	p value*
First born child	14 (25%)	26 (44.8%)	**0.027**
Dual hereditary	46 (82.1%)	38 (65.5%)	**0.044**
Maternal asthma	17 (30.4%)	11 (19.0%)	0.158
Paternal asthma	15 (26.8%)	13 (22.4%)	0.588
Sibling asthma	18 (32.1%)	10 (17.2%)	0.065
Maternal smoking during pregnancy	7 (12.5%)	15 (26.3%)	0.064
Maternal allergy	41 (73.2%)	39 (67.2%)	0.486
Paternal allergy	31 (55.4%)	32 (55.2%)	0.984
Sibling allergy	34 (60.7%)	28 (48.3%)	0.183
Cord IgE > 0.5	14 (35.9%)	18 (39.1%)	0.759

Figures are numbers (percentages) of participants. *Pearson's Chi-square test,

4.6 Objective markers of asthma in the participants seen at 18-year follow-up

We compared spirometry, bronchial hyper-responsiveness, and markers of airway inflammation, exhaled nitric oxide (FeNO) and inflammatory cell counts from induced sputum, between those with, and without a diagnosis of asthma irrespective of group. In addition we compared the prevalence of atopy between those with asthma and those without.

4.6.1 Spirometry and Bronchial hyperresponsiveness

There was no significant difference in spirometry between those with and without asthma, although there was a trend, albeit non-significant for all parameters of lung function to be less amongst the asthmatics compared to the non-asthmatics. The dose response slope was significantly higher amongst the asthmatics compared to the non-asthmatics and this translates into significantly more bronchial hyper-responsiveness in those with asthma (**Table 4.4**).

Table 4.4. Comparison of Spirometry and Bronchial hyper-responsiveness in those with and without asthma

% Predicted	No asthma (n=82) (95% C.I)	Asthma (n=20) (95% C.I)	*p value
FEV_1	98.73 (96.32-101.14)	93.99 (86.96-101.02)	0.11
FVC	97.02 (94.43-99.6)	95.09 (87.78-102.4)	0.54
FEV_1/FVC	102.25 (100.45-104.05)	99.64 (94.44-104.84)	0.24
PEFR	96.53 (92.75-100.3)	96.97 (93.56-100.39)	0.59
DRS (Transformed)	0.76 (0.66-0.86)	1.22 (0.90-1.54)	**0.01**

Figures represent means (95% confidence interval) *One-way ANOVA

4.6.2 Airways inflammation and atopy in those with versus without asthma

Eosinophils from induced sputum were significantly higher in those with asthma (**Table 4.5**.). A general linear model was used to compare the level of FeNO amongst asthmatics compared to non-asthmatics as we have previously shown that smoking is a significant factor in reducing the level of FeNO[181]. FeNO was significantly higher in those with asthma compared to those without asthma, 29 ppb (95% C.I, 23-39) versus 21ppb (95% C.I, 18-24), $p = 0.024$. Significantly more asthmatics compared to non-asthmatics were atopic 80.9% versus 39% ($p < 0.001$).

Table 4.5.Comparison of inflammatory cells in those with and without asthma

%	No asthma (n=11) Median (IQR)	Asthma (n=32) Median (IQR)	*p value
Epithelial cells	4.5 (1.9-9.25)	3.8 (2.63-5.55)	0.922
Neutrophils	9 (2.55-21.8)	2.8 (0.65-13.15)	0.077
Macrophages	80.5 (67.15-91.9)	80.3 (78.55-92.15)	0.504
Eosinophils	0.5 (0-1.3)	2.5 (1.15-4.25)	**0.007**
Lymphocytes	0 (0-0.15)	0 (0-0)	0.269

Figures represent medians (interquartile range) *Mann-Whitney independent samples U Test

4.7 Primary outcome: The prevalence of asthma at 18-year follow-up

In the unadjusted analysis there was a significant difference between the prevention 6/56 (10.7%) and control 15/58 (25.9%) groups in terms of the prevalence of asthma at 18 (p = 0.037, Pearson's Chi Square).

To assess whether factors other than grouping were significant in asthma at 18 year follow-up logistic regression was undertaken First

born status, dual heredity of allergic disease, male gender, passive smoking, exposure to pets and high cord blood IgE have all been considered as risk factors for asthma. For passive smoking we created the variable from maternal or paternal smoking in the first 2 years of life and pet exposure was either cat or dog present in the house in the first two years of life (no distinction was made between cat and dog in the first follow-up so it was impossible to separate this variable further). None of these factors were found to be significant (**Table 4.6.).**

Table 4.6. Early life risk factors and odds of asthma at 18- year follow-up

Variable	Odds Ratio for asthma (95% C.I)	*p value
Dual heredity	1.71 (0.33-8.90)	0.52
First born status	2.03 (0.63-6.57)	0.24
Male gender	1.99 (0.65-6.12)	0.23
High cord blood IgE	1.47 (0.33-6.57)	0.61
Passive smoking	1.47 (0.47-4.54)	0.51
Pet exposure	1.13 (0.35-3.64)	0.84

Figures represent odds ratios (95% confidence intervals)

*Binary logistic regression,

A logistic regression model, backward (LH) method, was constructed to assess the influence of these factors and group. Group remained the sole significant variable with an odds ratio for asthma in the prevention group of 0.26 (0.08 – 0.88 95% C.I), p = 0.030.

4.7.1 Asthma phenotypes

Asthma in childhood is variable in terms of onset, remission and relapse and this variability may well reflect different phenotypes of asthma. We therefore classified our participants into those who never had asthma at any follow-up (never asthma), those who had asthma at 18-year follow-up and at a previous follow-up (persistent asthma), those with current asthma but no asthma at any previous follow-up (new onset asthma), and those without current asthma but had asthma at a previous follow-up (remitted asthma). The prevention group were significantly less likely to have persistent asthma and were more likely to never had have asthma (**Table 4.7**).

Table 4.7. Asthma phenotypes at 18-year follow-up

Variable	Prevention n=56	Control n=58	p-value*
Never asthma	32	22	**0.04**
Persistent asthma	4	13	**0.02**
New onset asthma	2	2	0.97**
Remitted asthma	18	21	0.70

*Pearson's chi-square, **Fisher's Exact Test.

A multinomial logistic regression model was constructed using never asthma as the reference category with group as a factor. The risk of persistent asthma was significantly higher for the control group compared to the prevention group odds ratio 4.73 (1.36-16.42 95% C.I), $p < 0.05$. There was no significant difference seen for new onset

asthma or for those in remission. The factors dual heredity and first born status were added to the model to assess whether these factors were significant, neither of these additional factors were significant and group remained significant within this model.

4.7.2 Atopy and asthma

There was a significant difference in atopic and non atopic asthma between the groups, (**Table 4.8**.). When we looked at each group separately the control group had significantly more participants with atopic asthma. In contrast there was no significant difference between atopic and non atopic asthma in the prevention group.

Table 4.8. The prevalence of atopic asthma at the 18-year follow-up.

Variable	Non-atopic asthma	Atopic asthma	p value*
Control	2/15 (13.3%)	13/15 (86.7%)	**0.046**
Prevention	2/6 (33.3%)	4/6 (66.7%)	
Control	2/15 (13.3%)	13/15 (86.7%)	**0.002**
Prevention	2/6 (33.3%)	4/6 (66.7%)	0.38**

Figures represent numbers (percentage) of participants.

*Pearson's Chi Square **Fisher's Exact Test.

The odds ratio of atopy for asthma at 18 was assessed for all participants, and then was modelled separately for the prevention and control groups with, and without adjustment for firstborn status and dual heredity. Adjusting for first born and dual heredity did not alter the

findings that whilst atopy was a significant risk factor for asthma for the study population as a whole and for the control group, it was not a significant factor for the prevention group (**Table 4.9**).

Table 4.9. The risk of atopy for asthma at 18

Variable	Odds ratio atopy for asthma (95% C.I)	p value*
Overall (n=103)	6.25 (1.89 -20.62)	0.003
Prevention (n=48)	2.90 (0.46-18.31)	0.260
Control (n=55)	10.56 (2.02-55.14)	0.005

Figures represent odds ratios (95% confidence interval).

*Binary logistic regression model, adjusted for dual heredity and firstborn status.

4.8 Secondary Outcomes at 18-year follow-up

4.8.1 Asthma Severity

4.8.1.1 Asthma Quality of life questionnaires (AQLQ)

A total of 21 participants had asthma and consequently answered quality of life questionnaires. Overall there was no significant difference between the two groups. There was a significant difference in the activities related domain with the prevention group reporting significantly more activity related symptoms compared to the control group (**Table 4.10**).

Table 4.10. AQLQ scores.

Variable**	Prevention n=6 Median (IQR)	Control n=15 Median (IQR)	*p value
AQLQ	5.1 (4-6.1)	6.01 (5.6-6.5)	0.073
AQLQ-Symptoms	4.7 (3.4-5.9)	5.7 (5.1-6.3)	0.112
AQLQ-Activities	5.5 (4.5-6.5)	6.4 (6.1-6.7)	**0.018**
AQLQ-Emotional	5.1 (3.4-6.7)	6.2 (5.5-6.8)	0.095
AQLQ-Environment	5.2 (4.2-6.2)	5.8 (5.3-6.3)	0.267

Figures represent medians (interquartile ranges). *Mann-Whitney U test (2 tailed)

** The lower the score the greater the symptoms

4.8.1.2 Asthma related treatment requirements

There were no significant differences between the groups, in terms of treatment requirements (**Table 4.11**).

Table 4.11 Comparison of asthma treatment needs between groups

	British Thoracic Society asthma treatment steps**		*p value
	BTS 1 n (%)	BTS 2 + BTS 3 n (%)	
Prevention (n=6)	2 (33%)	4 (67%)	0.27
Control (n=15)	9 (60%)	6 (40%)	

Figures are numbers (percentages) of participants. *Pearson Chi square.

**BTS 1 = no treatment or short acting beta agonist. BTS 2 = short acting beta agonist and inhaled corticosteroid, BTS 3 = step 2 plus additional prophylactic agent.

No participant with asthma was on BTS Step >3

4.9 Spirometric markers of lung function

A total of 102 of the 103 participants seen in person underwent spirometry, 1 person attending the centre had recently undergone surgical repair of a persistent pneumothorax and could not tolerate forced expiratory manoeuvres. Spirometric results were stratified by atopy and asthma status to assess whether sub-analysis by atopic and asthma status would be feasible (**Table 4.12**.). Due to the small numbers involved such sub-analysis was not possible.

Table 4.12. Atopic and asthma status of participants who underwent spirometric testing

	Asthma	Atopy		Total	p value*
		Yes	No		
Prevention	No	17 (35%)	24 (50%)	48**	>0.05
	Yes	4 (8%)	2 (4%)		
Control	No	15 (28%)	25 (46%)	54	>0.05
	Yes	13 (24%)	1 (2%)		

Figures present numbers (percentages) of participants.

*Fisher's exact test 2 sided

**1 participant declined skin prick testing, therefore atopic status unknown.

Overall there was no significant difference in spirometry between the groups. Specifically, there was no significant difference in the lung function of those with asthma in the prevention versus the control group. There was a non-significant trend for the participants with atopy (without asthma) in the prevention group to have higher FEV_1 values compared to the atopic controls, and a significantly higher FEV_1/FVC ratio (**Table 4.13**).

Table 4.13. Comparison of spirometry between groups.

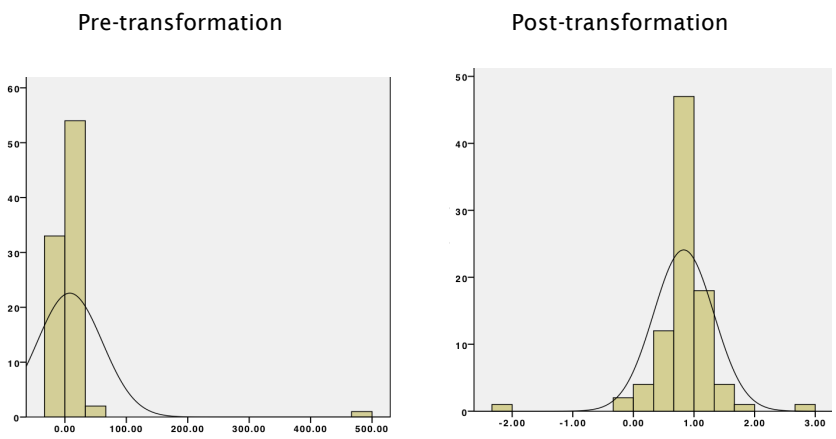
	Prevention mean (SD)	Control mean (SD)	*p value
All Participants	**n = 48**	**n = 54**	
FEV_1 % predicted	96.87 (24.12)	98.63 (10.90)	0.460
FVC	95.22 (12.48)	97.90 (12.60)	0.283
FEV_1/FVC	102.59 (9.72)	100.97 (7.99)	0.358
PEFR	95.89 (19.50)	97.99 (14.89).	0.544
Asthma only	**n = 6**	**n = 14**	
FEV_1	85.67 (17.39)	97.55 (12.96)	0.106
FVC	91.80 (12.43)	96.50 (17.03)	0.552
FEV_1/FVC	94.71 (16.23)	101.75 (7.95)	0.202
PEFR	95.15 (21.77)	100.88(17.24)	0.533
No Asthma	**n = 42**	**n = 40**	
FEV_1	98.47 (11.72)	99 (10.25)	0.826
FVC	95.71 (12.56)	98.39 (10.87)	0.304
FEV_1/FVC	103.72 (8.11)	100.7 (8.08)	0.096
PEFR	95.1 (19.51)	97.1 (14.22)	0.773
Atopic, no Asthma	**n = 17**	**n = 15**	
FEV_1	102.44 (12.08)	99.66 (10.72)	0.499
FVC	96.1 (10.91)	100.3 (11.15)	0.291
FEV_1/FVC	106.67 (6.72)	99.62 (6.80)	**0.006**
PEFR	100.89 (21.75)	93.99 (12.83)	0.304
No atopy, No asthma	**n = 24**	**n = 25**	
FEV_1	96.12 (10.97)	98.61 (10.16)	0.413
FVC	96.09 (13.67)	97.25 910.76)	0.742
FEV_1/FVC	101.41 (8.52)	101.35 (8.83)	0.981
PEFR	93.38 (17.5)	98.84 (14.90)	0.244

Figures represent means (standard deviations). *Independent T Test.

4.10 Bronchial hyperresponsiveness

A total of 92 participants underwent methacholine challenges, 44 in the prevention group, and 48 in the control group. Only three participants achieved a positive PC_{20}, which did not enable meaningful analysis to be performed using this variable. Bronchial hyperresponsiveness (BHR) was analysed as a continuous variable, the dose response slope (DRS), as % decline in FEV_1/total cumulative dose methacholine. BHR was not normally distributed (**Figure 4.2**) and contained negative as well as positive numbers. In order to transform the variable to a normal distribution the largest negative value plus 0.01 (to counter a value of 0) was added as a positive number (+6.54) to the data which was then transformed to its Log_{10}

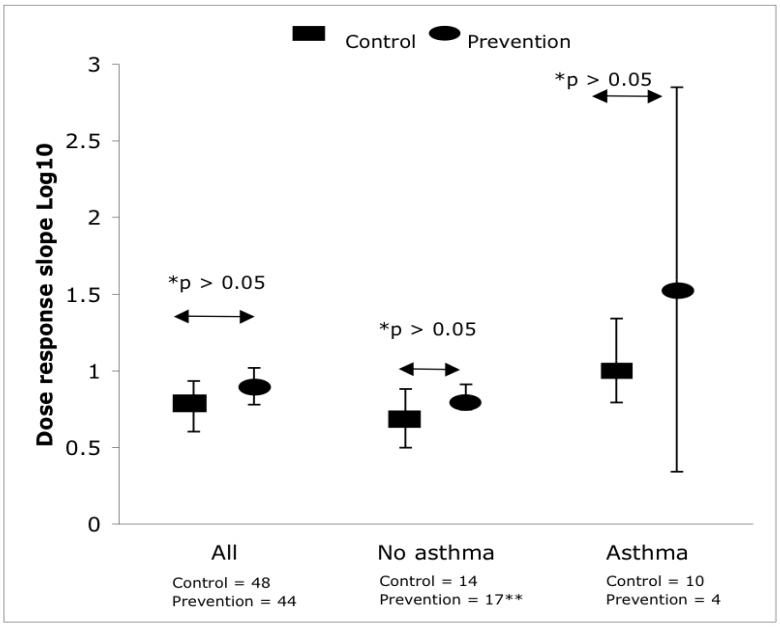
Figure 4.2. Histogram of the DRS pre and post transformation



There was no significant difference between the prevention and control groups in terms of BHR overall, and in those with and without asthma (**Figure 4.3**.). Due to the small numbers involved it was impossible to stratify asthma by atopic status (one non-atopic person with asthma

underwent methacholine challenge in each group). Comparisons in those without asthma by atopic status did not demonstrate a significant difference between the groups.

Figure. 4.3. Comparison of BHR at 18 between the prevention and control groups.



Markers represent mean dose response curve with lines representing the 95% confidence interval for each.

*ANOVA. ** 1 participant in the prevention group refused skin prick testing.

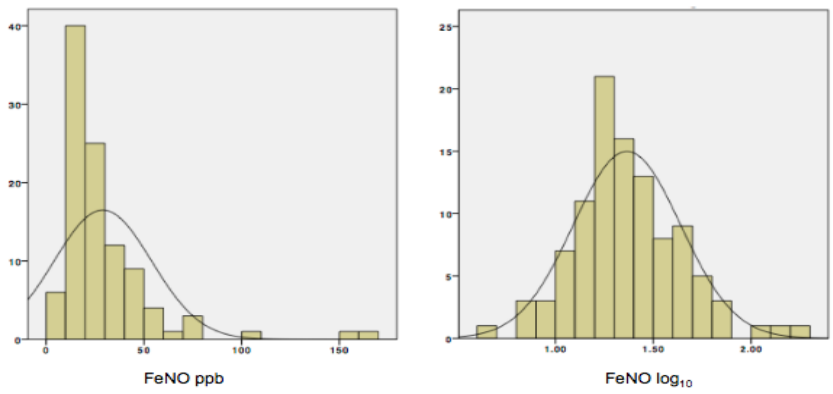
4.11 Airways inflammation

4.11.1 Exhaled Nitric Oxide (FeNO)

FeNO was measured in 103 of the participants, 49 of the prevention group and 54 of the control group. FeNO ppb was found to be non-normally distributed (Shapiro-Wilk, $p < 0.001$) and was transformed to its Log_{10} (**Figure 4.4**) to ensure a normal distribution (Shapiro-Wilk, $p > 0.05$), for the purposes of reporting FeNO was back-transformed to its geometric mean.

Figure 4.4. Histograms of FeNO ppb and FeNO log_{10}

Pre-transformation Post-transformation



There was no significant differences between the groups overall, and when comparing the groups by atopic and asthma status (**Table 4.14**),

although the trend was for the control group to have higher levels of FeNO. A general linear method (GLM) using ANOVA was used to adjust for the impact of smoking, atopy, and asthma; there were no significant differences between the groups (p = 0.167), although the control group had slightly higher levels of FeNO at 23 ppb (95% C.I, 18-29) compared to the prevention group 20 ppb (95% C.I, 16 - 26).

Table 4.14. Comparison of FeNO ppb levels between the prevention and control groups.

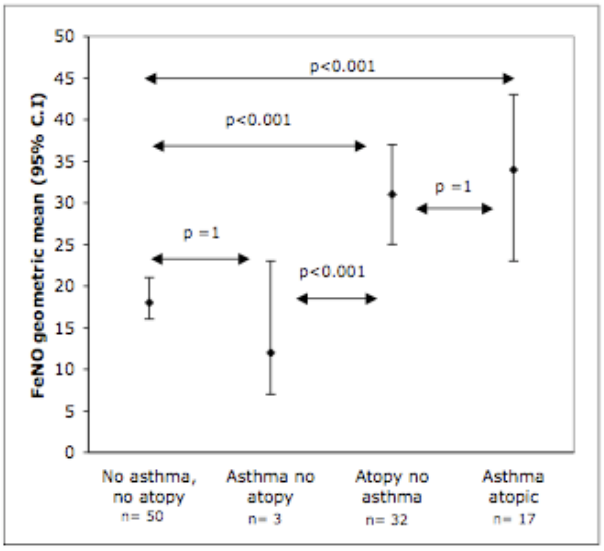
Variable	n	Prevention FeNO ppb geometric mean (SD)	n	Control FeNO ppb geometric mean (SD)	*p value
All	49	21 (2)	54	25 (2)	0.167
Asthma	6	21(2)	14	34 (2)	0.184
No asthma	43	22 (2)	40	23 (2)	0.599
Atopy	17	28 (1)	15	34(2)	0.457
No atopy	25	18 (1)	25	18 (2)	0.894

Numbers represent geometric means (SD). *Independent T Test.

4.11.1.1 FeNO and the influence of atopy

Atopy was a significant factor in the level of FeNO, once atopic status had been taken into account asthma was not significant in the absence of atopy (**Figure 4.5**).

Figure 4.5. Atopy and FeNO



Generalised Linear Model (ANOVA)

4.11.2 Airway inflammatory cells

A total of 52 participants underwent sputum induction, 24 from the prevention group and 28 from the control group. 43 viable sputum samples were obtained, 19 (79%) from the prevention group and 24 (86%) from the control group. There was no significant difference in viable samples between the two groups (p = 0 .716, Fisher's exact test). We were unable to obtain viable sputum samples from participants who were non-atopics with asthma (**Table 4.15**). Data pertaining to sputum cell counts was not normally distributed, therefore non-parametric methods were used to analyse the data.

Table 4.15. Successful sputum induction by atopy and asthma status.

	Prevention		Control		p value*
	Asthma	No asthma	Asthma	No asthma	0.89
Atopic	4	5	7	5	
Non-atopic	0	10	0	12	

*Pearson's Chi-square

Overall there was a non-significant trend for epithelial cells and eosinophils to be elevated in the control group compared to the prevention group, but no significant differences were found (**Table 4.16**). Eosinophils, neutrophils and epithelial inflammatory cell counts were not significantly different between the prevention and control participants with asthma (**Table 4.16**). Epithelial cells were higher in the control group and this difference just missed achieving significance. The small numbers of participants does limit analysis somewhat, as whilst the trend is for less inflammatory cells in the prevention group compared to the control group further analysis is not rewarding.

Table 4.16. Inflammatory cell counts in participants with, and without asthma,

% Cell counts	Prevention	Control	*p value
All	n = 19	n = 24	
Epithelial cells %	2.8 (1.15-6.75)	5.3 (2.9-9.9)	0.08
Neutrophils %	9.5 (2.9-16.15)	5.65 (1.55-15.75)	0.470
Macrophages %	86.5 (77.4-92.4)	79.55 (67.15-91.4)	0.235
Eosinophils %	0.5 (.15-1.9)	0.8 (.3-4.75)	0.408
Lymphocytes %	0	0 (0-.15)	0.466
Asthma	n =4	n=7	
Epithelial cells %	2.15 (0.9 – 3.55)	4.8 (3.65 -13.050	0.059
Neutrophils %	1.9 (0.9 – 9.150	2.8 (.4 – 13.15)	0.776
Macrophages %	92.9 (86.15 94.3)	78.8 (73.55 – 85.65)	**0.047**
Eosinophils %	3 (1.75 – 3.5)	1.8 (1.05 – 5.88)	1
Lymphocytes %	0	0	0.45
No asthma	n=15	n=17	
Epithelial cells %	2.8 (1.55-9.25)	5.8 (2.8-8.5)	0.365
Neutrophils %	10.3 (4.15-19.3)	5.8 (1.8 – 23.8)	0.509
Macrophages %	85 (73.9-91)	80.5 (66-91.8)	0.558
Eosinophils %	0.5 (0- 1.15)	0.5 (0-2.8)	0.564
Lymphocytes %	0	0 (0-0.3)	0.546
Atopic, no asthma	n = 5	n = 5	
Epithelial cells %	1 (0.8 -2)	7.3 (5.8 -8.3)	0.142
Neutrophils %	3 (0.8 -5)	5.8 (2.8 – 48.3)	0.465
Macrophages %	92.8 (88.5 -96.5)	71.8 (44.5 – 80.5)	**0.047**
Eosinophils %	1.3 (0.3 – 1.3)	4.5 (0.3 – 9.5)	0.343
Lymphocytes %	0	0	1
Non-atopic, no asthma	n=10	n=12	
Epithelial cells %	4.5 (2.3 – 11)	4.3 (2.55 – 10.15)	1
Neutrophils %	16.05 (10.3 21.8)	7.15 (1.8 -19.4)	0.086
Macrophages %	77.4 (64-86.5)	81.65 (67.15 92.8)	0.356
Eosinophils %	0.4 (0-0.5)	0.4 (0 -1.05)	0.683
Lymphocytes %	0	0 (0-0.3)	0.495

Figures represent median percentage cell count (95% confidence interval). *Mann-Whitney U test

4.12 Atopy at 18-year follow-up

102 of the 114 participants underwent skin prick testing. Of the 12 who did not, 10 participated via telephone questionnaire only, whilst 1 participant refused to undergo skin prick testing due to needle phobia, and 1 participant had IgE specific blood tests (all negative). There was a non-significant trend for the prevention group to have a lower prevalence of atopy, HDM sensitization and food allergen sensitisation compared to the control group (**Table 4.17**).

Table 4.17: Comparison of results of skin prick tests, prevention versus control

	Prevention (n=48)	Control (n =54)	p value*
Histamine	48 (100.0%)	53 (98.1%)	0.343
Saline	0 (0.0%)	0 (0.0%)	n/a
House dust mite	14 (29.2%)	23 (42.6%)	0.159
Cat	9 (18.8%)	16 (29.6%)	0.202
Dog	10 (20.8%)	11 (20.4%)	0.954
Grass	17 (35.4%)	14 (25.9%)	0.298
Tree	8 (16.7%)	9 (16.7%)	1
Cladosporium	3 (6.3%)	4 (7.4%)	1 **
Alternaria	3 (6.3%)	6 (11.1%)	0.495 **
Milk	4 (8.3%)	2 (3.7%)	0.416**
Egg	0	1 (1.9%)	1**
Wheat	9 (18.8%)	6 (11.1%)	0.277
Peanut	3 (6.3%)	3 (5.6%)	0.882**
Cod	2 (4.2%)	2 (3.7%)	1**
Soya	2 (4.2%)	0	0.219**
Atopy at 18***	21/48 (44%)	28/55 (51%)	0.468
Aero-allergens***	21/48 (43.8%)	28/55 (50.9%)	0.468
Food allergens***	9/48 (18.8%)	8/55 (14.5%)	0.566

Figures represent numbers (percentages) of participants. *Pearson's Chi square. ** Fishers exact test *** One participant underwent IgE specific blood tests

We looked at timing of onset of atopy and house dust mite, other aeroallergens and food allergens, categorising them into persistent, those who were sensitised at both 8 and 18 year follow-up and those who were new onset – sensitised at 18 year follow-up (**Table 4.18**). We found that there was a significant difference in persistent atopic sensitisation with significantly less persistent sensitisation in the prevention group. This difference appeared to be due to significantly less persistent sensitisation of the prevention group to house dust mite (**Table 4.18**).

Table 4.18. Persistent versus new-onset atopy, and sensitisation at 18-year follow-up

	Prevention n =47†	Control n=54†	p value*
Persistent atopy (atopy 8 and 17)	9 (19%)	22 (41%)	**0.01**
New onset atopy (no atopy at 8, atopy at 17)	12 (26%)	6 (11%)	0.06
Persistent HDM (sensitised at 8 and 18)	5 (11%)	17(32%)	**0.01**
New onset HDM (sensitised after 8 years)	9 (19%)	6 (11%)	0.62
Persistent aeroallergens (sensitised at 8 and 18)	8 (17%)	13 (24%)	0.38
New onset aeroallergens (sensitised after 8 years)	10 (21%)	9 (17%)	0.11
Persistent Food sensitisation	1 (2%)	0 (0%)	0.28**
New onset Food sensitisation	9 (19%)	8 (15%)	0.56

Figures represent numbers (percentages) of participants. *Pearson's Chi Square. **Fisher's exact test

† Participants who underwent skin prick testing at 8 and 18-year follow-up.

A regression model was constructed to assess the influence of early life factors and group on atopy. Within this model no factors were significant (**Table 4.19**)

Table 4.19 Group and early life factors and atopy

Variable	Odds Ratio for atopy (95% C.I)	*p value
Group	0.64 (0.23-1.80)	0.40
Dual heredity	3.41 (0.67-17.46)	0.14
First born status	2.56 (0.89-7.57)	0.81
Male gender	1.27 (0.49-3.31)	0.62
High cord IgE	1.90 (0.45-8.00)	0.61
Passive smoking	0.76 (0.28-2.05)	0.59
Pet exposure	0.56 (0.19-1.62)	0.28

*Binomial logistic regression

4.13 The prevalence of eczema at 18-year follow-up

12.5% (7/56) of the prevention group versus 25.9% (15/58) of the control group had eczema at 18. This difference was not significant p =0.071 (Pearson Chi-Square test). A logistic regression model was constructed to assess the impact of early life factors and group within this model there was a significant difference for group (**Table 4.20**).

Table 4.20. Group and early life factors and eczema at 18-year follow-up,

Variable	Odds Ratio for eczema (95% C.I)	*p value
Group	0.18 (0.04-0.86)	**0.03**
Dual heredity	5.99 (0.82-43.62)	0.08
First born status	0.94 (0.23-3.79)	0.93
Male gender	1.39 (0.40-4.88)	0.61
High cord IgE	5.32 (0.90-31.47)	0.07
Passive smoking	1.55 (0.44-5.48)	0.50
Pet exposure	0.37 (0.10-1.39)	0.14

*Binomial logistic regression model,

A backward (LH) logistic regression model was constructed to further assess this finding, when dual heredity and a high cord IgE were present the prevention group had a significantly lower likelihood of eczema OR 0.23 (0.05-0.93 95% confidence intervals) p = 0.04). No other factors were found to be significant in this model.

There was no significant difference in the prevalence of atopic versus non-atopic eczema in the control group compared to the prevention group 10/15 (66.7%) versus 4/7 (57.1%), p = 1.00. Significantly less participants in the prevention group developed eczema compared to the control group (**Table 4.21**).

Table 4.21 Age of onset of eczema

Variable	Prevention n =56	Control n =58	p value*
New-onset eczema	2/7 (29%%)	11/15 (73%)	0.02
Persistent eczema	5/7 (71%)	4/15 (27%)	0.74

Figures represent numbers (percentages) of participants.

*Fisher's exact test

4.13.1 Eczema severity at 18-year follow-up

There was no significant difference between the two groups in terms of the overall SCORAD score, or in terms of the extent of eczema, intensity of eczema or subjective symptoms (**Table 4.22**).

Table 4.22. SCORAD Score

	Prevention n =5 Median (IQR)	Control n=6 Median (IQR)	*p value
SCORAD total	22.12 (20.78-27.80)	18.65 (12.58-61.28)	0.715
% of body area	0.60 (0.40-1.40)	1.7 (0.40-2)	0.462
Intensity of rash	6 (6-7)	4.50 (3-12)	0.521
Subjective symptoms	3 (3-6)	3.5 (0-18)	0.854

Figures represent the median (interquartile range) SCORAD score.

*Mann Whitney U Test

4.14 The prevalence of rhinitis at 18-year follow-up

There was no significant difference in the prevalence of rhinitis at 18-year follow-up between the prevention (21/35) and the control groups (20/38), p = 0.74 (Pearson's Chi-square), this remained the case after adjusting for early life factors (**Table 4.23**). Within this model however firstborn status, dual heredity significantly increased the likelihood of rhinitis.

Table 4.23. Early life factors, group and rhinitis

Variable	Odds Ratio for rhinitis (95% C.I)	*p value
Group	0.69 (0.22-2.16)	0.52
Dual heredity	6.91 (1.15-41.54)	**0.04**
First born status	8.72 (2.64-28.78)	**<0.001**
Male gender	0.75 (0.26-2.14)	0.59
High cord blood IgE	1.90 (0.45-8.00)	0.61
Passive smoking	0.43 (0.14-1.33)	0.14
Pet exposure	0.63 (0.20-2.02)	0.44

* Binomial logistic regression

There was no significant difference between the two groups in terms of atopic and non-atopic rhinitis, or age of onset and persistence of rhinitis (**Table 4.24**).

Table 4.24 Atopic and non-atopic rhinitis and age of onset of rhinitis

	Prevention n =56	Control n=58	*p value
Atopic rhinitis	15	15	0.44
Non atopic rhinitis	5	3	0.20
Persistent rhinitis	6	6	0.92
New onset rhinitis	15	14	

Figures represent numbers (percentages) of participants. *Pearson Chi-Square Test

4.14.1 Rhinitis intermittent versus persistent, and severity

There were no significant differences between the groups in terms of persistence of rhinitis symptoms (**Table 4.25**).

Table 4.25. Intermittent versus persistent rhinitis

	Control	Prevention**	*p value
Intermittent rhinitis	11/20 (55%)	10/19 (52.6%)	0.882
Persistent rhinitis	9/20 (45%)	9/19 (47.4%)	

Figures represent numbers (percentage) of participants. *Pearson Chi-Square Test. **2 participants did not answer the questions

There were no significant differences between the two groups in terms of the severity of rhinitis (**Table 4.26**).

Table 4.26. Severity of rhinitis

	Control	Prevention**	*p value
Sleep disturbance	9/20 (45%)	8/19 (42.1%)	0.855
Interfered with daily activities	14/20 (70%)	13/19 (68.4%)	0.915
Interfered with work	12/20 (60%)	13/19 (68.4%)	0.584
Any impairment	16/20 (80%)	14/19 (73.7%)	0.640

Figures represent numbers (percentages) of participants. * Pearson Chi-Square test. **2 participants did not answer the questions

4.15. Summary

In the unadjusted analysis there was a significant difference between the prevention and control groups in terms of the prevalence of asthma at 18, with the prevention group having a significantly lower prevalence of asthma.

To assess whether factors other than grouping were significant in asthma at 18 year follow-up logistic regression was undertaken. First born status, dual heredity of allergic disease, male gender, passive smoking, exposure to pets and high cord blood IgE were assessed, none of these factors were found to be significant.

Asthma in childhood is variable in terms of onset, remission and relapse and this variability may well reflect different phenotypes of asthma. We therefore classified our participants into those who never had asthma at any follow-up (never asthma), those who had asthma at 18-year follow-up and at a previous follow-up (persistent asthma), those with current asthma but no asthma at any previous follow-up (new onset asthma), and those without current asthma but had asthma at a previous follow-up (remitted asthma). The prevention group were significantly less likely to have persistent asthma and were more likely to never had have asthma compared to the control group.

There was a significant difference in atopic and non atopic asthma between the groups. When we looked at each group separately the control group had significantly more participants with atopic asthma. In contrast there was no significant difference between atopic and non atopic asthma in the prevention group.

In terms of asthma severity overall there were no significant differences between the two groups in terms of asthma quality of life questionnaires, asthma treatment requirements or markers of airways inflammation or bronchial hyperresponsiveness.

We looked at timing of onset of atopy and house dust mite, other aeroallergens and food allergens, categorising them into persistent, those who were sensitised at both 8 and 18 year follow-up and those who were new onset – sensitised at 18 year follow-up. We found that there was a significant difference in persistent atopic sensitisation with significantly less persistent sensitisation in the prevention group. This difference appeared to be due to significantly less persistent sensitisation of the prevention group to house dust mite

There was no significant difference in the prevalence of eczema or rhinitis in the control or prevention groups. Nor were there significant differences in the severity of eczema or rhinitis between the two groups.

Chapter 5: Longitudinal analysis from 1 to 18 years

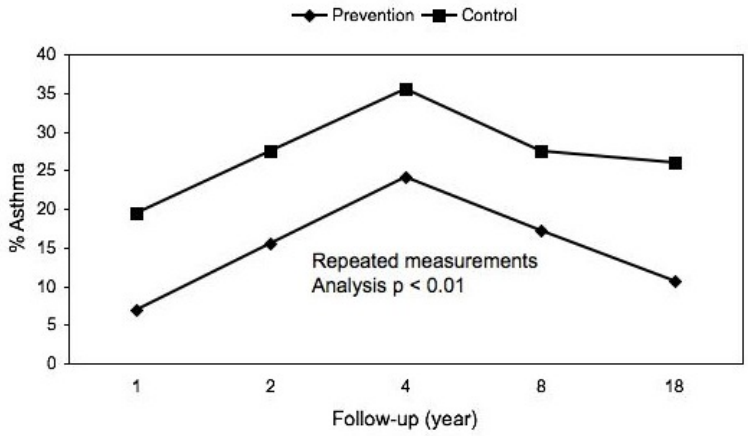
5.1 Introduction

The period prevalence of primary and secondary outcomes have been analysed over the length of the follow-up, from 1 to 18 years using generalized estimating equations (GEE) to provide repeated measures analysis.

5.2 The period prevalence of asthma

Over the length of follow up, the period prevalence of asthma was significantly less in the prevention group compared to the control group (**Figure 5.1**).

Figure 5.1. The period prevalence of asthma over follow-up.



GEE repeated measures analysis, unadjusted.

GEE models were constructed to adjust for the impact of early life risk factors on the period prevalence of asthma. The effect of dual heredity, firstborn status, pet exposure, maternal and paternal smoking, gender and group were assessed, within this model the prevention group continued to have a significantly lower OR for asthma (**Table 5.1**).

Table 5.1. Early life risk factors, and the period prevalence of asthma.

Factor	Odds ratio for asthma	95% C.I	p value*
Prevention	0.51	0.32-0.81	**0.004**
First born	0.77	0.49-1.21	0.25
Dual heredity	1.33	0.82-2.18	0.25
Male gender	1.71	1.11-2.64	0.15
Pet exposure	0.92	0.59-1.43	0.72
Maternal smoking	1.36	0.81-2.28	0.24
Paternal smoking	1.78	1.05-2.99	**0.03**

*GEE repeated measures analysis.

The individual impact of maternal paternal and sibling asthma were examined (the variable dual heredity was removed). Maternal asthma was associated with a significantly increased odds ratio of asthma as was male gender. Belonging to the prevention group remained significantly protective against asthma (**Table 5.2**).

Table 5.2: Maternal asthma, male gender and the risk of asthma.

Factor	Odds ratio for asthma	95% C.I	p value*
Prevention	0.41	0.26-0.67	< **0.001**
Male gender	1.79	1.14-2.81	**0.01**
First born	0.67	0.40-1.13	0.13
Maternal asthma	3.23	1.97-5.30	< **0.001**
Paternal asthma	1.13	0.66-1.95	0.66
Sibling asthma	1.08	0.60-1.94	0.80
Pet exposure	0.90	0.58-1.39	0.63
Maternal smoking	1.18	0.67-2.07	0.58
Paternal smoking	1.44	0.81-2.57	0.21

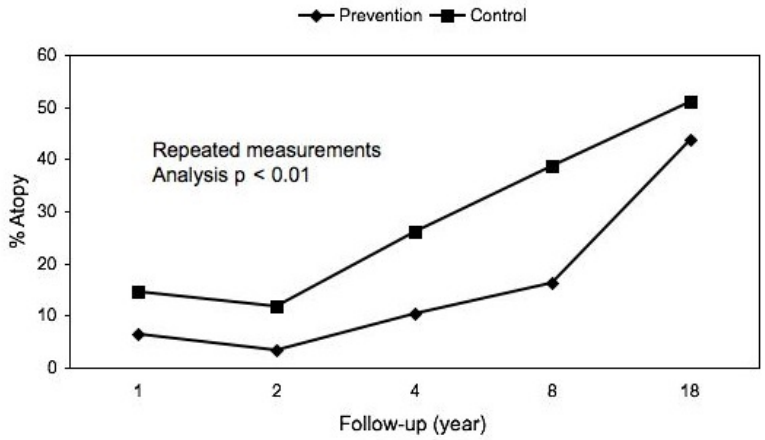
*GEE repeated measures analysis.

5.3 Longitudinal analysis of atopy

5.3.1 The period prevalence of atopy

Over the length of follow up there was a significantly lower period prevalence of atopy in the prevention group compared to the control group (**Figure 5.2**). The difference between the 2 groups was noticeable from the earliest follow-up at 1-year post intervention and persisted throughout the follow-up, although the difference had narrowed at 18-year follow-up.

Figure 5.2. The period prevalence of atopy over follow-up.



GEE repeated measures analysis, unadjusted.

Adjusting for early life factors did not alter the significant difference between the control and prevention groups in terms of atopy, belonging to the prevention group was significantly protective against atopy. Firstborn status, dual heredity and male gender significantly increased the likelihood of atopy onset (**Table 5.3**).

Table 5.3. Early life factors and the period prevalence of atopy.

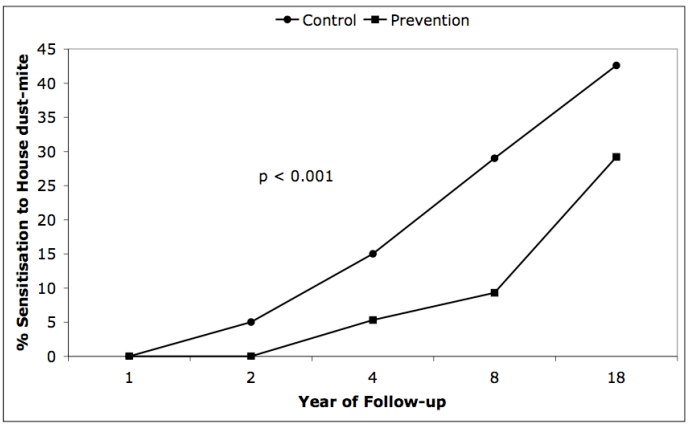
	Odds ratio for atopy	95% C.I	p value*
Prevention group	0.44	0.27-0.72	**0.001**
First born	1.95	1.22-3.14	**0.006**
Dual heredity	2.16	1.21-3.86	**0.01**
Male gender	1.74	1.10-2.73	**0.02**
Pets	1.36	0.88-2.10	0.17
Maternal smoking	0.78	0.45-1.36	0.39
Paternal smoking	1.58	0.92-2.72	0.09

*GEE repeated measures analysis

5.3.2 The period prevalence of house dust-mite

Over the length of the follow-up there was a significant difference in the prevalence of house dust-mite sensitization with the prevention group having significantly less sensitisation to house dust-mite (**Figure 5.3).**

Figure 5.3 the period prevalence of sensitisation to house dust-mite

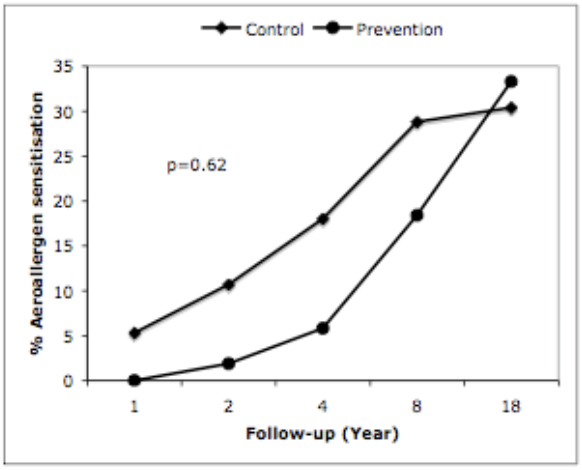


GEE repeated measures analysis, unadjusted.

5.3.3 The period prevalence of aeroallergen sensitisation, excluding HDM

Over the length of the follow-up there was not a significant difference between the 2 groups in terms of sensitisation to aeroallergens (cat, dog, grass, tree pollen, alterneria and cladosporium) other than house dust-mite (**Figure 5.4**).

Figure 5.4 The period prevalence of aeroallergens (excluding HDM)



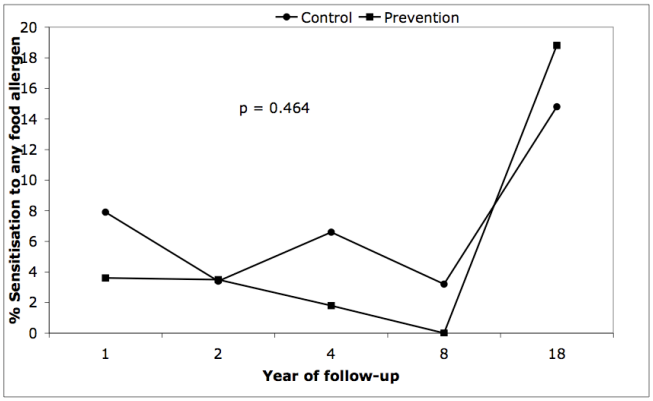
GEE repeated measures analysis, unadjusted.

When we looked at sensitization up to, and including 8 year follow-up there was a significant difference, with the prevention group significantly less likely to be sensitised to aeroallergens (other than HDM) 0.39 CI 0.19-0.77, p =0.007.

5.3.4 The period prevalence of food sensitisation

Over the length of the follow-up there was no significant difference between the groups in terms of the prevalence of food allergen sensitisation (**Figure 5.5**).

Figure 5.5. The period prevalence of sensitisation to food allergens.

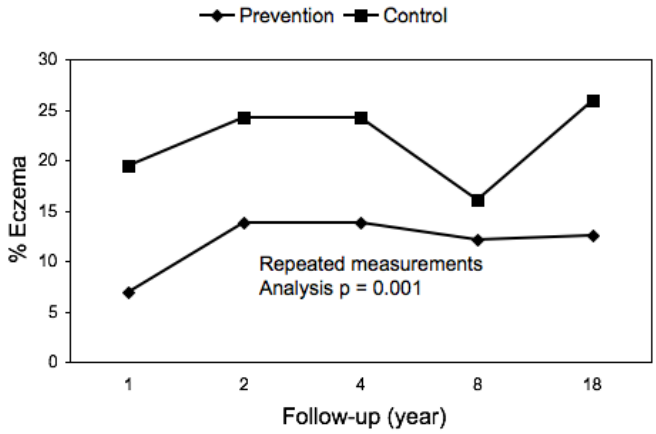


GEE repeated measures analysis, unadjusted.

5.4 Prevalence of eczema over the length of follow-up

The prevention group had a significantly lower period prevalence of eczema over the length of follow-up compared to the control group (**Figure 5.6**).

Figure 5.6. The period prevalence of eczema over follow-up



GEE repeated measures analysis, unadjusted.

In adjusted analysis the prevention group had a significantly lower OR for eczema compared to the Control group (**Table 5.4**.) No other factors analysed were found to be significant.

Table 5.4. Adjusted Odds ratio for eczema over the length of follow-up

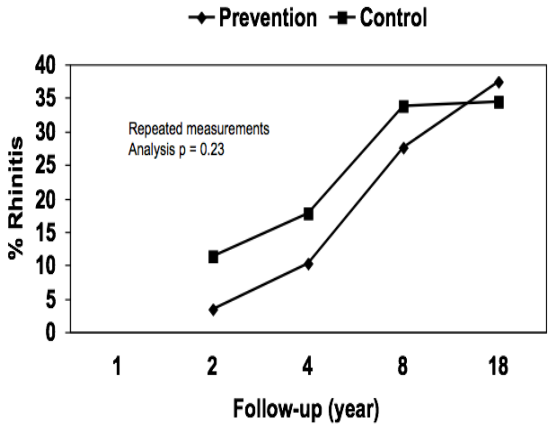
	Odds ratio for eczema	95% C.I	p value*
Prevention group	0.31	0.16-0.59	**0.001**
First born	0.76	0.46-1.24	0.27
Dual heredity	1.09	0.64-1.84	0.76
Male gender	0.68	0.43-1.08	0.10
Pets	1.21	0.75-1.94	0.43
Maternal smoking	0.48	0.27-0.84	**0.01**
Paternal smoking	0.68	0.43-1.08	0.10

*GEE repeated measures analysis

5.5 Period prevalence of rhinitis over the length of follow-up

In unadjusted analysis there was no significant difference in the period prevalence of rhinitis between the two groups (**Figure 5.7**).

Figure 5.7. Prevalence of rhinitis over follow-up



GEE repeated measures analysis, unadjusted.

In adjusted analysis group was not a significant factor but first-born status and male gender were significant risk factors for rhinitis onset (**Table 5.5**).

Table 5.5. Adjusted Odds ratio for rhinitis over the length of follow-up

	Odds ratio for rhinitis	95% C.I	p value*
Prevention group	0.29	0.15-0.61	0.32
First born	1.94	1.17-3.23	**0.01**
Dual heredity	1.42	0.78-2.58	0.25
Male gender	1.96	1.21-3.18	**0.007**
Maternal smoking	0.64	0.35-1.17	0.15
Paternal smoking	0.92	0.49-1.69	0.78
Pets	0.98	0.61-1.65	0.98

*GEE repeated measures analysis

5.6 Period prevalence of any disease and early life factors

The prevalence of any disease in the total study population and by group at each follow up is shown in Table 5.6

Table 5.6. Prevalence of any allergic disease at each follow-up.

Year	Any disease present Total study population	Any disease present Control group	Any disease present Prevention group
1	27/120 (22.5%)	21/62 (33.9%)	6/58 (10.3%)
2	42/120 (35%)	29/62 (46.8%)	13/58 (22.4%)
4	52/120 (43.3%)	33/62 (53.2%)	19/58 (32.8%)
8	54/120 (45%)	31/62 (50%)	23/58 (39.7%)
18	56/114 (49.1%)	31/58 (53.4%)	25/56 (44.6%)

Any disease included rhinitis, eczema and/or asthma.

The period prevalence of any disease (rhinitis, eczema and/or asthma) was significantly lower in the prevention group compared to the control group, even after adjusting for the potential impact of other early life factors (**Table 5.7**).

Table 5.7. Risk of any disease over the length of follow-up

	Odds ratio for any disease	95% C.I	p value*
Prevention group	0.48	0.33-0.69	**<0.00**
First born	0.92	0.63-1.36	0.68
Dual heredity	1.23	0.81-1.86	0.34
Male gender	1.44	1.01-2.06	**0.04**
Maternal smoking	0.96	0.62-1.51	0.87
Paternal smoking	1.57	1.01-2.45	**0.04**
Pets	0.51	0.62-1.28	0.51

*GEE repeated measures analysis

5.7. Summary:

Over the length of follow up, the period prevalence of asthma was significantly less in the prevention group compared to the control group. GEE models were constructed to adjust for the impact of early life risk factors on the period prevalence of asthma. The effect of dual heredity, firstborn status, pet exposure, maternal and paternal smoking, gender and group were assessed, within this model the prevention group continued to have a significantly lower OR for asthma.

Over the length of follow up there was a significantly lower period prevalence of atopy in the prevention group compared to the control group. The difference between the 2 groups was noticeable from the earliest follow-up at 1-year post intervention and persisted throughout the follow-up, although the difference had narrowed at 18-year follow-up. Adjusting for early life factors did not alter the significant difference between the control and prevention groups in terms of atopy, belonging to the prevention group was significantly protective against atopy. Over

the length of the follow-up there was a significant difference in the prevalence of house dust-mite sensitization with the prevention group having significantly less sensitisation to house dust-mite. Over the length of the follow-up there was not a significant difference between the 2 groups in terms of sensitisation to aeroallergens (cat, dog, grass, tree pollen, alterneria and cladosporium) other than house dust-mite. When we looked at sensitization up to, and including 8 year follow-up there was a significant difference, with the prevention group significantly less likely to be sensitised to aeroallergens, other than HDM. Over the length of the follow-up there was no significant difference between the groups in terms of the prevalence of food allergen sensitisation.

The prevention group had a significantly lower period prevalence of eczema over the length of follow-up compared to the control group. There was no significant difference in the period prevalence of rhinitis between the two groups. The period prevalence of any disease (rhinitis, eczema and/or asthma) was significantly lower in the prevention group compared to the control group, even after adjusting for the potential impact of other early life factors.

Chapter 6: Discussion

6.1 Introduction

This randomised controlled study examined the effects of a dual intervention in the first year of life on the prevalence of asthma at 18 years. The hypothesis was that environmental manipulation to reduce exposure to house dust mite and dietary allergens in individuals at high risk of developing asthma would reduce the prevalence of asthma at 18 years of age.

6.2 Principal Findings

6.2.1 Primary outcome asthma prevalence

At the 18-year follow-up, the prevalence of asthma was significantly reduced in the prevention group (10.7%) compared to the control group (25.9%). Thus our hypothesis that dual intervention in the first year of life in participants at high risk of developing asthma would reduce asthma prevalence was confirmed. Data from the 18-year follow-up of the Isle of Wight birth cohort study was also reviewed. The overall prevalence of asthma was 17.7% (231/1305). When we reviewed the prevalence of asthma in participants with dual heredity 24.3% (26/81) had asthma. Although participants from the 18-year birth cohort study are from a different study, what is marked is that the prevention group have a noticeably lower prevalence of asthma compared to the 18-year birth cohort study overall but also specifically when compared to those participants in the 18-year study with dual heredity. In contrast the control group have a similar prevalence to those participants with dual heredity in the 18-year birth cohort. This does support our hypothesis

that the dual intervention was successful in reducing the prevalence of asthma in this high-risk group of individuals.

When asthma was categorised in terms of timing of onset we found that persistent asthma was significantly more likely in the control group and the prevention group were significantly less likely to ever have had asthma (**Table 4.7**). This implies that firstly the intervention successfully reduced the likelihood of asthma developing in the prevention group, and as there was significantly more persistent asthma in the control group that the intervention achieved its effect in early childhood. Longitudinal analysis showed that the significant difference between the groups for asthma was present over the entire length of follow-up (**Figure 5.1**) implying that the intervention exerted its effect in early childhood.

6.3 Secondary outcomes

6.3.1 Atopy

The rationale for our intervention was that by allergen avoidance we would reduce the impact of atopy as a driver for asthma onset. We found that there were significantly more participants with atopic asthma in the control group compared to the prevention group (**Table 4.8),** this suggests that the intervention was successful by attenuating the interaction between atopy and the individual's predisposition to asthma onset.

Further support for this as a likely explanation for our interventions success comes from looking at the timing of onset of atopy (**Table 4.18**), as we found that like asthma there were significant differences between the groups in terms of whether participants were atopic from early childhood (persistent), or became sensitised between 8 and 18

year follow-up (new onset). The control group was significantly more likely to have atopy from early childhood, and this persistence was mainly due to persistent house dust-mite sensitisation.

In cross sectional analysis there was no significant difference in the prevalence of atopy between the groups at 18-year follow-up. When we looked at atopy over the length of follow-up there was a significant difference overall (**Figure 5.2**) and again house dust-mite sensitisation seemed to be the most significant individual allergen over the entire length of follow-up (**Figure 5.3**). Interestingly when we looked at aeroallergens (excluding house dust-mite) from 1 to 8 year follow-up we found there was significantly less sensitisation in the prevention group odds ratio 0.39 (0.19-0.77 95% C.I) p <0.001. Implying that between 8 and 18 year follow up the significance was lost due to an increased number of participants in the prevention group developing aeroallergen sensitivities.

An explanation for how the intervention in early life achieved a reduction in asthma may therefore be that by delaying the onset of atopy to later on in childhood an interaction between atopy and predisposition to asthma during a critical period in early childhood was avoided. In a recent publication[190] we constructed a binary logistic regression model of the interaction between atopy and group as the sole explanatory variable at 18 but this was not significant, but given the small numbers involved this is not surprising.

6.3.2 Reduction in asthma severity

Definitions of what is meant by asthma severity vary considerably in the literature [191], as asthma may be severe in terms of lung function, BHR, exacerbations and treatment needs but be well controlled. Equally symptoms may be relatively mild but control may be poor resulting in frequent exacerbations and health care attendances. There is broad

agreement that asthma severity can be assessed by the individual's treatment requirements and their degree of control [192–195]. We have assessed asthma control using the Juniper asthma quality of life questionnaire and the individual's treatment requirements based on the British Thoracic Society treatment steps.

All 21 participants with asthma answered the AQLQ. There were no significant differences in participants in terms of their overall quality of life with reference to their asthma, and consequently overall control was not significantly different between the two groups (**Table 4.10**). Looking at individual subcategories within the AQLQ, responses to symptoms, emotional and environmental domains based questions were not significantly different between the two groups. The prevention group reported significantly more symptoms related to activities compared to the control group. It is difficult to know whether this is a chance finding or intriguingly this may suggest that the prevention group had more participants with a particular phenotype of asthma where exercise was a more common trigger.

There was no significant difference in terms of asthma treatment needs between the two groups (**Table 4.11**), with the majority of participants with asthma being in either treatment group 1 (no treatment/ short acting beta agonist) or treatment group 2 (Beta agonist plus prophylactic medication).

6.3.3 Spirometric lung function and bronchial hyperresponsiveness

We did not find a significant difference between the participants with or without asthma in the prevention and control groups in terms of spirometric measures of lung function (**Table 4.13**) or in terms of BHR (**Figure 4.3**). This is consistent with us finding no difference between

the two groups in terms of severity of asthma. Additionally the lack of difference between the two groups in terms of objective markers of asthma suggests that the intervention globally reduced asthma prevalence, rather than reducing asthma at the mild or severe end of the spectrum. Our results are consistent with the CCAPS study, which at 7-year follow-up did not find a significant difference in lung function or BHR[158].

6.3.4 Reduction in markers of airways inflammation, FeNO and induced sputum

Our prevention study is the first multifaceted intervention study to assess and analyse non-invasive markers of airways inflammation. We did not find a significant difference between the groups in terms of their FeNO (**Table 4.14**), whether or not the analysis was adjusted for asthma, smoking status and inhaled corticosteroid use, although there was a non-significant trend for FeNO to be less in the prevention group compared to the control group.

When we stratified all the participants into atopic and non-atopic irrespective of the intervention grouping (**Figure 4.5**), we found that, whilst atopy was a significant factor on the level of FeNO, asthma in the absence of atopy was not. This suggests that FeNO is heavily influenced by atopic status rather than asthma *per se* as reported by us previously[181]. Given the small number of participants with asthma in each group the effect of atopic versus non-atopic status was lost when comparing the prevention and control groups.

Amongst the participants with asthma we were limited in only achieving viable samples in those who were atopic (**Table 4.15**). There were less epithelial cells in those with asthma in the prevention group compared to the control group (**Table 4.16**), although this did not achieve

statistical significance (p = 0.059). There were however, significantly less macrophages in the prevention group (p < 0.05). There were no significant differences in eosinophils, neutrophils or lymphocytes between the groups with asthma. Given the small numbers involved and lack of non atopic subjects, it is difficult to speculate further.

Overall we did not find major evidence of significant differences in airways inflammation between the two groups. These findings are likely to be hampered by the small numbers and the lack of inclusion of non-atopic asthmatics, as in a larger study combining sputum samples from the prevention study and the Isle of Wight birth cohort study we found that atopy was associated with significantly higher numbers of eosinophils, and when we compared atopic and non atopic asthmatics atopy was the driver for increased eosinophils rather than asthma per se[196].

6.3.5 Reduction in eczema

In unadjusted analysis there was a non-significant reduction in the prevalence of eczema (7 prevention versus 15 control, p = 0.071). In terms of onset of eczema (**Table 4.21**) there were significantly less participants in the prevention group developing new onset eczema, suggesting that the intervention reduced the risk of developing eczema in later childhood. In a logistic regression model in the presence of dual heredity and high cord blood IgE the prevention group had a significantly lower risk of eczema odds ratio 0.23 (0.05-0.93 95% C.I) p = 0.04.

In longitudinal analysis, the period prevalence of eczema was significantly lower in the prevention group compared to the control group (**Figure 5.6**). Our results certainly support the view that environmental manipulation is able to reduce the prevalence of eczema

in high-risk populations, though not to such an extent as we have demonstrated for asthma. The lack of statistical significance on unadjusted analysis may be due to the small sample size; equally it is possible that there are differences in the interactions between genetic predisposition and environmental manipulation for eczema compared to asthma. For example in cross sectional analysis in our study dual heredity appears to be a significant risk factor for eczema onset but is not a significant factor for asthma

6.3.6 Reduction in rhinitis

At the 18-year follow-up, we did not find a significant difference in the prevalence of rhinitis, persistence or new onset of rhinitis or atopic rhinitis between the prevention and control group (**Table 4.23**). Nor did we find that the two groups differed in terms of the persistence of symptoms or the severity of rhinitis (**Table 4.24**). When we compared the period prevalence of rhinitis between the two groups over the length of the follow-up group was not a significant factor, although male gender and first-born status were (**Table 5.5**). Rhinitis therefore does not seem as amenable to intervention as asthma and eczema. This may reflect pathophysiological and natural history differences; it is possible that in both skin and the airway symptoms are usually dependent on a degree of chronicity, whereas in rhinitis symptoms are directly attributable to acute inflammation in response to exposure to allergens and other triggers. Also, onset of asthma and eczema is often traced back to early childhood while onset of rhinitis peaks in adolescence and by that time the atopy prevalence was similar in the two groups.

6.3.7 Early life factors and atopy, asthma, eczema and rhinitis

We examined whether there were early life factors that were significantly associated with asthma, eczema and rhinitis in order to firstly ensure that the outcomes we were seeing were due to being in the prevention or control group, and secondly to see if any other factors increased or decreased the likelihood of disease onset and were significant alongside group. Identifying other significant factors would enable us to identify subgroups that were more or less likely to benefit from the intervention.

Cross sectional analysis at 18 year follow-up did not demonstrate that early life factors were significant in the onset of asthma at 18 (**Table 4.6**). Importantly, given that despite randomisation dual heredity was significantly higher in the prevention group and first-borns were more common in the control group, neither of these factors were significantly associated with an increased risk of asthma. Longitudinal analysis did show that maternal asthma significantly increased the odds of asthma onset over the length of follow-up (**Table 5.2**), and this is consistent with other studies assessing heredity and asthma risk. The significance of maternal asthma may not be apparent in cross sectional analysis because over the length of the study asthma remits and relapses and so cross-sectional analysis is likely to 'miss' a proportion of participants with asthma and hence not find an association between early life factors and asthma risk whereas longitudinal analysis will detect such associations.

The odds ratio for the period prevalence of atopy was significantly increased by dual heredity, first born status and male gender, although group still remained significant (**Table 5.3**). This is consistent with other studies assessing atopy risk[146]. Aside from group in longitudinal analysis maternal smoking appeared to exert a protective effect for eczema onset odds ratio 0.48 (0.27-0.84 95% C.I). Other studies have noted that smoking exposure exerts a variable effect in terms of

reducing or increasing risk of allergic disease depending on timing of exposure[197] and perhaps individual genetic factors. First-born status was a significant factor in longitudinal analysis for increased rhinitis risk and this has been identified as a significant risk factor for rhinitis previously[92].

No other early life factor other than group was consistently and significantly associated with disease onset, and the small numbers involved does mean these findings are likely to be speculative rather than authoritative. It is possible however that maternal asthma may be a stronger risk of asthma than dual heredity – and using this to select potential participants for future interventions may prove rewarding.

6.4 Strengths and limitations of this study

6.4.1 Power of the study

The small sample size is a draw back in this study. Firstly, the small sample size means that the precision of the results is limited with there being large confidence intervals. Secondly, the small sample size means that the power of the study to detect small or moderate differences between the groups is relatively low. We were able to confirm the study's primary hypothesis. In terms of secondary outcomes, it is possible that in a larger sample many of the borderline significant findings would have become significant and would have moved the discussion away from being speculative to authoritative. In terms of attempting to assess which factors in early life may have a significant bearing on risk of asthma onset and response to the intervention, again small number has limited analysis. We have tried to overcome some of these issues with a longitudinal analysis where the additional data points can increase the robustness of the analysis.

6.4.2 Study design

Our study was not double-blinded and therefore methodologically was suboptimal. However, from a practical point of view, it was impossible to conceal house dust mite avoidance measures from the participants and their families, equally concealing dietary intervention would have been impossible. At each follow-up the researchers assessing the participants were blinded to the original grouping of the children and thus bias on the part of the researchers was minimised. Given many of our outcome measures were objective, or could be supported by objective markers, bias on the part of participants would have a minimal effect on our study.

Whilst the literature suggests that single interventions have largely been unsuccessful in reducing asthma onset[161], a factorial design (comparing groups divided into control, HDM avoidance, Dietary avoidance and HDM plus dietary intervention) would enable this finding to be proven or disproved.

6.4.3 Recruitment

We were able to achieve 96% (n = 114/120) follow-up 18 years after the initial intervention. In contrast other intervention studies have not been as successful. At 2 year follow-up the PREVASC study achieved 93%[157] (n = 443/476) follow-up. The CAPPS 7 year follow-up was 69.7%[158] (n = 380/545) and the ACAP saw 60%[159] (n = 370/616) of their participants at 5 year follow-up. Our study had the lowest attrition of any study. This is likely to be due to the unique nature of our study centre it is on an island with a relatively stable population. The centre is a charitable trust that has very close ties with the local community, and

a policy of disseminating the results of the studies to the participants and their families after each follow-up.

Whilst it is possible that those who were not seen at 18 year follow-up may have the potential to alter the significance of this study, this is less likely given the small number lost to follow up (n = 6) and previous follow-up from this study where all 120 were seen and showed at various time points significant differences in both primary and secondary outcomes[165,198–200]. Not all of our participants were able to attend the study centre. A total of 12 either underwent telephone questionnaires (n = 9) or M.S visited them at home (n=3). It is possible that this may have affected our results. However none of the participants who answered the telephone questionnaire had asthma (the primary end point) and M.S was able to perform skin prick testing, spirometry and measure FeNO in the participants visited, 2 of whom were diagnosed with asthma.

6.4.4 Demographics

In the 18-year study follow-up participants were well matched between the two groups in terms of gender, family income, education, smoking status and exposure, and pet exposure (**Table 4.2**.). In terms of early life risk factors for asthma, there were significant differences in first born status and dual heredity (**Table 4.3**) between the two groups but neither of these factors were significant in the primary outcome of asthma at 18 years.

6.4.5 Assessment methods

6.4.5.1 Lung function testing and bronchial challenge

We did not specifically measure spirometric reversibility at any stage of the follow-up and this may have provided additional information on the likelihood of the presence of airway remodelling. Balanced against this, is keeping the number of tests and the time taken to do them to a minimum to avoid exhausting the participants and keeping the cost of the study as low as possible. Short acting beta agonists were avoided by participants prior to spirometry, and bronchial challenge, but we did not ask our participants to stop inhaled corticosteroids or long acting beta agonists. It is possible that given their long mode of action neither spirometry nor methacholine challenge reflected participants 'true' results. The difficulty with asking participants to stop all prophylactic asthma medication would be increasing their risk of relapse/exacerbations and given our primary aim was to assess the prevalence of asthma rather than its severity it would have been difficult to justify asking participants to omit their long acting preventers to an ethics committee.

6.4.5.2 Food allergy assessment

A significant criticism in terms of assessing the prevalence of food allergy is the difficulty in distinguishing allergy from intolerance, as well as distinguishing between IgE mediated versus non-IgE mechanisms (for example salicylate and sulphite sensitivity). We did not feel able to comment on the prevalence of true IgE mediated food allergy due to the lack of objective evidence in terms of food challenges and serum

specific IgE. In a larger study more in depth questionnaires and oral challenges may be appropriate.

6.4.5.3 Genetic markers

Given the dramatic advancements made in molecular biology over the last two decades the lack of collection of genetic samples prior to 18 year follow-up in this study is regrettable. Serial collections and assessment of DNA especially in the early years may have potentially identified important interactions between the intervention and genetic changes thus identifying mechanisms underlying this intervention

6.4.6 Defining asthma

In our study asthma was defined as a positive response to the question "have you wheezed in the last 12 months" and a positive response to "has a doctor diagnosed you with asthma". Where a participant had not reported wheezing episodes in the last year to be defined as asthmatic they had to be on prophylactic asthma medication.

In clinical trials objective markers of asthma are generally required – the lack of BHR is often used as an exclusion criteria. Our study definition of asthma may therefore be critiqued for the lack of such a marker. There are however, a number of rebuttals that can be made. Firstly, there is ample epidemiological evidence that BHR is a common finding in the general population with a prevalence of 10-30%[60]. Secondly, BHR is not seen in all individuals with asthma, with studies in populations with established asthma reporting a prevalence of 43%.[201] Thirdly, BHR is not fixed, BHR may decrease[202] and increase,[203] as well as

remit[204] and relapse[205] over an individuals lifetime. So whilst BHR does have a higher prevalence in asthma populations these three associated points mean that an asthma definition dependant on the presence of BHR runs the risk of misclassification.

A questionnaire-based definition of asthma has been extensively validated in epidemiological studies[56,70,206–208]. Indeed studies comparing questionnaire based diagnosis versus bronchial hyperresponsiveness have found the questionnaire to be either equivalent[70] or superior to BHR[56] in successfully diagnosing asthma when compared to the 'gold standard' of physician diagnosed asthma.

We did compare BHR and markers of airway inflammation between participants with and without asthma. We found that BHR was significantly higher amongst those participants with asthma (**Table 4.4**). Regarding markers of airway inflammation, exhaled nitric oxide (FeNO) and sputum eosinophils were significantly higher in those with asthma (**Table 4.5**) than those without asthma. These objective findings support our definition of asthma.

6.5 Comparisons with other multi-factorial primary prevention studies

Our study is unique, as it is the first dual intervention study to have followed up the participants from birth to early adulthood. Comparing our findings to other studies assessing the impact of dual intervention is therefore difficult as asthma is a disease that both remits and relapses in childhood[209], and varies in the age of onset[210]. Therefore, follow-up at different time points in childhood may differ in point prevalence of asthma, and this may not necessarily reflect the true effect of an intervention. Assessing the prevalence of asthma in early

life may result in misclassification as epidemiological studies have shown that one third of children wheeze before their third birthday but less than 40% of these go on to develop persistent symptoms.[22] Other intervention studies differ in their selection of candidates and interventions used[155,157,211]. Nonetheless, our finding of a significant reduction in asthma using the dual intervention of house dust-mite (HDM) avoidance and dietary modification is not an isolated finding.

6.5.1 Successful multi-factorial intervention studies

The PREVASC Study 1 and 2 year follow-up

The Prevention of Asthma in Children (PREVASC) study consisted of four interventions: 1) HDM avoidance, 2) Pet allergen avoidance, 3) Food allergen avoidance by breast-feeding for the first 6 months of life (supplemented/substituted where necessary with an extensively hydrolysed formula) and 4) Delayed introduction of solid foods after the age of 6 months. There was a significant reduction in HDM, cat and dog allergen levels at 1 year in the intervention group, and significantly more of the intervention group were exclusively breast-fed, and underwent delayed introduction of solid foods compared to the control group[212]. At the 2-year follow-up, there was no significant difference in total IgE, or specific IgE for HDM, cat or dog. In terms of outcome, the study found that there was a significant reduction in parental reporting of asthma symptoms at two years, and a non-significant trend of less G.P recorded morbidity over the length of the follow-up[157].

PREVASC study 6 year follow-up

At two years of age the children in the intervention arm were

randomised into two further groups 1) Extended intervention group (n=111) where reduction in HDM exposure and exposure to tobacco smoke continued until 4. 2) Short intervention group (n = 108) where the interventions stopped at 2. The control group (n=221) continued to be followed-up. Atopy was not reported to have been reassessed between 2 and 6-year follow-up. A minimum number of 194 infants per group were required to detect a reduction in asthma incidence by at least 30% (Type I error 0.05 and power of 0.80)[160]. The study found no difference in the main outcome of asthma at 6 years of age when both intervention groups were combined and compared to the control group. Analysis of the subgroups in the intervention arms was underpowered but no significant differences in asthma were found when compared to the control group. The reported prevalence of wheeze and nighttime coughing in the extended treatment arm approached significance ($p = 0.085$ and $p = 0.060$). It is difficult to know if the lack of significant findings at 6 years is because the original intervention's effectiveness was simply not effective longer term, whether separation of the intervention group into two further arms led to the study being underpowered to find significant differences in the two intervention groups. Or, whether the further interventions from 2 to 6 years may have impacted on the original interventions success, this is a possibility given the abundance of literature on timing of exposure playing an important role in whether a factor is protective, the inverse or ineffective[197].

The Canadian asthma primary prevention study

The Canadian Childhood asthma primary prevention study (CAPPS) consisted of the following interventions: 1) HDM avoidance, 2) During the last trimester of pregnancy and during lactation mothers were advised to avoid nuts and seafood, 3) cat and dog avoidance in the home, 4) Environmental tobacco smoke (ETS) avoidance in the home, 5)

Breast-feeding for at least 4 months (partially hydrolysed formula substituted/supplemented where necessary), 6) Delayed introduction of solids until 6 months – and advised to avoid introducing dairy products, nuts and seafood for the first year of life, 7) Advised to avoid day care for the first year of life[213]. The intervention group had significantly lower levels of HDM allergen at 12-month follow-up, and significantly lower cat allergens in the home at 4 months. There was no difference in exposure to ETS. Significantly more infants in the intervention group underwent prolonged breast-feeding and delayed introduction of solids and avoided day care[213].

At 7 year follow-up CAPPS reported a significant reduction in the prevalence of paediatrician diagnosed asthma in their intervention group compared to their control group (14.9 versus 23%)[158]. In this age group, a diagnosis of asthma is more certain, and thus demonstrates that our findings are not in isolation. A significant difference between the CAPP study and our own is atopic sensitization. We found that there was significantly less atopic asthma, and over the length of our study the period prevalence of atopy was significantly different between the prevention and control group. In contrast the CAPP study found no difference between the groups in terms of atopy or atopic asthma[158]. In sub-analysis the CAPP study found that in those who developed atopy after 1 year of age, the intervention was significantly associated with reduced OR of asthma (adjusted OR, 0.26; 95% CI, 0.08-0.88), but in those who had manifested atopy before or by 1 year follow-up the intervention did not significantly reduce the OR for asthma (OR, 0.68; 95% CI 0.20-2.22)[214]. The subgroup analysis supports the notion that delaying atopy onset impacts on asthma onset.

Atopic sensitisation in the CAPP study whilst significant in subgroup analysis did not appear to be as strongly associated with asthma onset

as in our study, it is possible that differences in selection criteria may have some bearing on this difference – we recruited participants on the basis of a family history of allergic disease and where applicable a high cord IgE, hence our study population were more at risk of atopic disease rather than asthma *per se*. In contrast the CAPPS selection criteria was either a first-degree relative with asthma or two first-degree relatives with atopic disease[213]. It is possible that this difference in selection may alter the interaction between atopy and asthma predisposition and the response to environmental manipulation, in other words atopy may not be such a driver of asthma pathogenesis in the CAPP study compared to our own. This may be a more likely explanation given that the CAPP study also acted to reduce exposure to environmental exposure to tobacco smoking and minimised pet exposure in the early years and this may have impacted on the risk of non-atopic participants for asthma onset.

6.5.2 Unsuccessful factorial studies

The Australian childhood asthma prevention study (ACAP) assessed the following interventions 1) HDM avoidance and 2) Increased proportion of omega-3 fatty acids in the diet and decreased proportion of omega-6 fatty acids, separately and in combination using a factorial design. Pregnant women whose unborn children were thought to be high risk of developing asthma (1 parent or sibling with current asthma or wheeze[215]) were recruited and randomised to 1 of four groups (active intervention or control groups for both HDM avoidance and dietary fatty acid modification). The primary outcome was a reduction in asthma. The study has reported outcomes at 3[215], 5[155] and 11.5[159] years. There was no significant difference in the prevalence of asthma between groups, nor was the combined intervention of HDM avoidance and dietary modification successful in reducing the prevalence of asthma at any stage of follow-up[155,159,215,216]. It is possible that the

differences in the choice of interventions in the ACAP study compared to the other studies may account for the lack of success of this intervention. Firstly the dietary intervention in the ACAP was primarily to increase the proportion of long chain polyunsaturated fatty acids in the diet and this contrast with the other studies where the dietary intervention consisted predominantly of avoidance of food allergens. Secondly the ACAP study did not encourage prolonged breast-feeding and/or use hydrolysed infant formula. Finally the ACAP study did not advocate-delayed introduction of solids in contrast to the other successful studies, this suggests that prolonged breast-feeding and/or delayed introduction of solids may be critical in the success of intervention studies.

6.6 Conclusions and implications for future research

Our study has successfully achieved its primary outcome of reducing the prevalence of asthma. We have also proven that the effects of such an intervention are long lasting. Thus we have proven our hypothesis that environmental manipulation may alter genetic predisposition to asthma. Repetition of this study on a larger scale is necessary for a number of reasons. Firstly, to ensure its validity, by replicating these findings. Secondly to enable detailed sub analysis to find out if there are significant early life factors that may alter the effectiveness of the interventions. Thirdly it is important to assess the cost-effectiveness of such an intervention before considering implementing it on a broader scale. Future research to assess which factors in the dietary intervention are the most important to success may enable a less strict avoidance strategy to be developed which will potentially improve dietary adherence and reduce the need for such intensive dietary monitoring during the intervention.

Appendices

Appendix 1 Participants information leaflets

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE
St Mary's Hospital, Newport, Isle of Wight, PO30 5TG. Tel: 01983 534898

PARTICIPANT INFORMATION SHEET –Isle of Wight Primary Prevention Study- 17/18 year follow-up

We would like to thank you for your involvement so far in the Isle of Wight Primary Prevention Study. The data we have been able to collect from you has been tremendously helpful in advancing our understanding of how to prevent asthma and other allergic diseases.

You are now being invited for a further follow-up as part of this study. Before you decide to participate it is important for you to understand why the research is being done and what it will involve.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?

Over the last few decades there has been an dramatic increase in the number of teenagers with asthma, eczema, hay fever and food allergy. We still do not know why people develop these diseases. The purpose of this study is to see if reduction to allergen exposure in infancy has any effect in reducing the number of teenagers with asthma and allergy. When we started this study i.e. in the first year of your life, some of you had reduced allergen exposure while others carried on normally. We also know that those with reduced allergen exposure did have less asthma and allergic diseases until the age of 8 when we last saw you. It is critically important to know if the preventive effect of

reduced allergen exposure continues in to adolescence. We will then be able to apply preventive measures for others who are born with a high risk of these disease e.g. those with a parent or older sibling with asthma.

Certain genes have been linked with the development of asthma and other allergic diseases. We need to know how much of the differences in asthma and allergy seen in the two groups are due to differing allergen exposure and how much due to differing genetic make-up. Some children with the same allergen exposure develop asthma and allergies whereas others do not. This depends on their genetic make-up. As some of you had reduced and others had normal allergen exposure in infancy, this gives us a unique opportunity to understand how genes interact with allergens to cause asthma and allergic diseases. If we are to maximise our chance of discovering how our genes are involved in the development of asthma and allergy, we need to look at the genes of as many of you as possible. We can do this by taking a small amount of blood. Alternatively we could collect a small amount of saliva from you.

Why have I been chosen?

You have been chosen because you are part of the Isle of Wight Primary Prevention Study. This is a birth cohort of one hundred and twenty young people, all born on the Isle of Wight in 1990 and 1991.

What will happen to me if I take part?

We will arrange for you to visit the David Hide Research Centre at a convenient time. We will reimburse your travel expenses. During this 2 hour visit we will repeat many of the procedures that you may have been asked to do at previous visits. We would like you to participate this time by undertaking all or part of the following:

- Questionnaire about your current health.
- Weight, height and blood pressure, and check whether you have signs of eczema or asthma.

- Collect a small amount of your breath to measure the amount of nitric oxide it contains. This tells us if you have inflammation in your lungs which will affect how they work.
- Spirometry – we will ask you to blow into a computer to check how well your lungs are working.
- Methacholine test – this is a special breathing test to ascertain how sensitive your lungs are. We will ask you to breathe in a mist containing methacholine and blow into a computer.
- Skin prick test – this is a safe, standard medical test for allergies (e.g. house dust mite); a drop of the liquid will be put on the skin and gently scratched; the test is positive if a small wheal develops after 15 minutes.
- We would like to take a small amount of blood using anaesthetic cream to numb the skin beforehand.
- If you are not happy to allow us to take some blood, we will ask for a saliva sample (we will ask you to spit into a small cup).
- Urine – we would ask you to provide us with a small urine sample.

What do I have to do?

We will ask you to come to the David Hide Asthma and Allergy Research Centre, at a convenient time, for up to a 2 hour visit. Before each visit we will ask you not to drink or eat any caffeine (eg coffee, tea, coke, chocolate) for 4 hours; if you take asthma medication please do not use your reliever inhaler (e.g. ventolin, salbutamol, terbutaline, bricanyl) for 6 hours; long acting inhaler (e.g. salmeterol, serevent, seretide, symbicort, eformoterol, oxis) for 12 hours; and antihistamines for 72 hours. If you have had a respiratory infection in the previous 2 weeks or are taking oral steroids we will rebook your visit at a convenient time for you.

What are the possible disadvantages and risks of taking part?

We will minimise any discomfort from taking blood with local anaesthetic cream to numb the skin. For some people the methacholine challenge may cause slight wheeze but this can be easily treated with an inhaler. In the highly unlikely event of any injury caused to you, medical care through the National Health Service will be available to you. St. Mary's Hospital NHS Trust has indemnified this study.

What are the possible benefits of taking part?

Your participation in this study is very important as it will allow us to see how asthma and allergies can be prevented in teenagers. The information we learn will help us to understand why children and teenagers develop asthma and other allergic problems and what can we do to prevent them. We can then form recommendations based on the results of this study. This is critically important as more of us are developing these illnesses.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. The first part of the consent form (Part A) asks for your consent to participate in the study. The second part (Part B) asks for permission to store any unused blood or other samples for use in future research into allergic diseases. The samples will only be used for studies approved by the Local Research Ethics Committee. The samples will be fully anonymous to the researchers who use them but contain codes that would allow the clinical study team who collected them to link them back to you. You are free to choose to just sign Part A and not sign Part B. You will receive a copy of the signed consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the medical care you receive.

What if there is a problem?

If you have any questions or concerns, please contact Dr Ramesh Kurukulaaratchy or Sharon Matthews at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534897. Email: preventionstudy@iow.nhs.uk.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential.

Contact for further information

Sharon Matthews or Ramesh Kurukulaaratchy, The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534897. Email: preventionstudy@iow.nhs.uk.

This completes Part 1 of the Information Sheet. Part 2 will give you more detailed information about the conduct of the study.

Part 2

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Sharon Matthews 01983 534897). If you still have questions or concerns, you can contact Alex Punter (Lead for Research and Development, St Mary's Hospital, Newport, Isle of Wight, PO30 5TG; email alex.punter@iow.nhs.uk).

In the very unlikely event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against St Mary's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

The personal information collected in this study will be kept confidential. The data we collect from you will not be labelled with your personal details and will be stored securely. Data collected during the study may be shared with our research collaborators in the USA; however they will not know who the information belongs to as your name and address will not leave The David Hide Asthma and Allergy Research Centre. Only the study personnel will have access to your personal details. You will not be individually identified in any reports or publications resulting from the study. We will keep your data on file for use in future studies approved by the Research Ethics Committee.

Who will have access to my health records?

Senior Investigators on this project will need to look at your health records to ensure safe conduct of the study procedures.

Involvement of the General Practitioner

We would like your permission to notify your General Practitioner (GP) of your participation in this study. With your permission we would send your GP the results of your allergy and methacholine tests as they may be useful for your future medical care. We would not send your GP any other results from the study.

What will happen to any samples I give?

Blood: we will use this to check how allergic you are. Additionally we will extract genetic material from the sample. This will only be used to look for genes that may be involved in asthma and allergies.

Urine: we plan to measure the level of cotinine in this sample, this increases if you have been exposed to cigarette smoke, passively or otherwise. The result will not be released to your parents or doctors.

Samples will be stored securely at the David Hide Asthma and Allergy Centre until they are analysed. Only the researchers at the centre will have access to them. Some of these blood (or saliva), and urine samples may be analysed outside of the St Mary's Hospital. The samples will not be labelled with your name or address so that the researchers analysing them will not know that the sample belongs to you. With your permission, we would like to store some blood (or saliva) and urine sample for use in further studies into asthma and allergic disease. We will only use stored samples for studies approved by the Local Research Ethics Committee.

Will any genetic tests be done?

As we explained in the "What is the purpose of the study?" section above, we are looking at which of our genes are involved in the development of asthma and other allergic diseases. For this work we can use blood or saliva samples. The results we obtain will help us to understand why some people develop asthma and allergies. The results will not directly help you and will not have any individual significance to you so we will not be able to give you your individual results.

What will happen to the results of the research study?

We aim to publish the results of the study in medical journals so that other doctors and researchers can make use of them. This is likely to be accompanied by an article in the local press on the Isle of Wight. It will not be possible to identify any individual teenager involved in the study from these published results.

Who is organising and funding the research?

Appendix 1

The researchers at The David Hide Asthma and Allergy Research Centre and university of Southampton are organising and carrying out this study. The study is being funded by the National Institute of Health Research, (patient benefit programme), which is part of the UK department of health

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Southampton and South West Research Ethics Committee B.

How long do I have to decide whether I should take part?

Your decision to participate in this study is entirely voluntary. You should take as much time as you need.

Thank you for taking time to read this information sheet.

Appendix 2 Participant Questionnaires

1) Questionnaire for all participants

1. ISAAC Questionnaire

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

PREVENTION STUDY	17/18 YEAR FOLLOW UP	STUDY NO

ISAAC QUESTIONNAIRE FOR ALL SUBJECTS

Date of birth ☐ Date seen ☐ Male ☐ Female ☐

I would like to ask you some questions about your home and family

1. Is your house Owned privately ☐ Rented privately ☐ Rented-council/house assn ☐ Other ☐

   Do you cook on Gas ☐ Electric ☐ Other ☐

2. Are you still living with your parents / guardians? Yes ☐ No ☐

3. Are you still in education? Yes ☐ No ☐

   If yes, are you in: School? ☐ 6th form college? ☐ Further education college? ☐

4. Do you work? Yes ☐ No ☐

   If yes, please specify: ☐

5. Father/mother's partner's occupation ☐

   Mother's occupation ☐

6. Birth order: child ☐ of ☐ (full sibs only) ☐ of ☐ (all mother's children)

7. Total annual family income (estimate)
   - Less than 12,000 ☐
   - 12,000 – 17,999 ☐
   - 18,000 – 29,000 ☐
   - 30,000 – 41,999 ☐
   - greater than 42,000 ☐

8. Does your home have damp spots on the walls or ceiling? Yes ☐ No ☐ Don't know ☐

9. Does your home have visible moulds or fungus on the walls or ceiling? Yes ☐ No ☐ Don't know ☐

10. SMOKING inside the house

Mother	Yes ☐	No ☐		/day
Father	Yes ☐	No ☐		/day
Other	Yes ☐	No ☐		/day

Outside the house (eg garden or porch) Yes ☐ No ☐

Other places Yes ☐ No ☐

11. PETS in last 2 years

Cat	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐
Dog	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐
Other	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐

1. **ISAAC Questionnaire**

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

PREVENTION STUDY	**17/18 YEAR FOLLOW UP**	**STUDY NO**			

ISAAC QUESTIONNAIRE FOR ALL SUBJECTS

Date of birth ☐ Date seen ☐ Male ☐ Female ☐

I would like to ask you some questions about your home and family

1. Is your house Owned privately ☐ Do you cook on Gas ☐
Rented privately ☐ Electric ☐
Rented-council/house assn ☐ Other ☐
Other ☐

2. Are you still living with your parents / guardians? Yes ☐ No ☐

3. Are you still in education? Yes ☐ No ☐
If yes, are you in: School? ☐ 6th form college? ☐ Further education college? ☐

4. Do you work? Yes ☐ No ☐
If yes, please specify: ☐

5. Father/mother's partner's occupation ☐
Mother's occupation ☐

6. Birth order: child ☐ of ☐ (full sibs only) ☐ of ☐ (all mother's children)

7. Total annual family income (estimate)
Less than 12,000 ☐
12,000 – 17,999 ☐
18,000 – 29,000 ☐
30,000 – 41,999 ☐
greater than 42,000 ☐

8. Does your home have damp spots on the walls or ceiling? Yes ☐ No ☐ Don't know ☐

9. Does your home have visible moulds or fungus on the walls or ceiling? Yes ☐ No ☐ Don't know ☐

10. SMOKING inside the house
Mother Yes ☐ No ☐ ☐/day
Father Yes ☐ No ☐ ☐/day
Other Yes ☐ No ☐ ☐/day

Outside the house (eg garden or porch) Yes ☐ No ☐
Other places Yes ☐ No ☐

11. PETS in last 2 years

Cat	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐
Dog	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐
Other	Yes including bedroom ☐	Yes, not bedroom ☐	Yes, not in house ☐	No ☐

STUDY NO ☐☐☐☐☐

12. How frequently are you annoyed by outdoor air pollution (from traffic, industry, etc.) in your home if you keep the windows open?

Every day ☐ Once a week ☐ Once a month ☐ Once a year ☐ Never ☐

13. How often do cars pass your house or on the street less than 100 metres away?

≥10 per hour ☐ 1-9 per hour ☐ 10 per day ☐ Seldom ☐ Never ☐

14. How often do heavy vehicles (e.g. trucks/buses) pass your house or on the street less than 100 metres away?

≥10 per hour ☐ 1-9 per hour ☐ 10 per day ☐ Seldom ☐ Never ☐

15. What term best describes the place you lived most of the time when you were under the age of five years?

Farm ☐ Rural village, eg Bembridge ☐ Small town, eg Ryde ☐ Suburbs of a city, eg Southampton ☐ City centre, eg Southampton ☐

16. Did your mother work on a farm while she was pregnant with you? Yes ☐ No ☐

17. Have you ever lived on a farm? Yes ☐ No ☐

If yes: When did you start living on a farm? ☐ When did you finish living on a farm? ☐

If yes, on the farm, did you have contact with live animals listed below:

Dairy cattle	Yes ☐	No ☐		Beef cattle	Yes ☐	No ☐	
Poultry	Yes ☐	No ☐		Sheep	Yes ☐	No ☐	
Pigs	Yes ☐	No ☐		Horses	Yes ☐	No ☐	
Other	Yes ☐	No ☐	Please specify				

If yes, did you spend your 1st year of life on a farm? Yes ☐ No ☐

If yes, is it an organic farm? Yes ☐ No ☐

18. Atopic disease since review at age 10 years

Asthma/wheezing episodes	Yes ☐	No ☐
Night time/recurrent cough (> 3 weeks)	Yes ☐	No ☐
Eczema	Yes ☐	No ☐
Year round/seasonal rhinitis *(Hay fever)*	Yes ☐	No ☐
Urticaria *(rash like nettle rash)*	Yes ☐	No ☐
Food intolerance or allergy	Yes ☐	No ☐

STUDY NO ☐☐☐☐

The following questions are about your chest

1. Have you ever had wheezing or whistling in the chest at any time in the past?
   Yes ☐ No ☐
   IF 'NO' SKIP TO Q.6

2. Have you had wheezing or whistling in the chest in the last 12 months?
   Yes ☐ No ☐
   IF 'NO' SKIP TO Q.6

3. How many attacks of wheezing have you had in the last 12 months?
   None ☐ 1-3 ☐ 4-12 ☐ >12 ☐

4. In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing
   Never woken with wheezing ☐ Less than one night per week ☐
   One or more nights per week ☐

5. In the last 12 months, has wheezing ever been severe enough to limit your speech to one or two words at a time between breaths? Yes ☐ No ☐

6. Have you ever had asthma? Yes ☐ No ☐
   If yes, was it physician diagnosed? Yes ☐ No ☐ Don't know ☐
   What age was it diagnosed? ☐☐ years of age

7. In the last 12 months, has your chest sounded wheezy during or after exercise?
   Yes ☐ No ☐

8. In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection? Yes ☐ No ☐

These questions are about your nose and eyes

9. Have you ever had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu? Yes ☐ No ☐
   IF 'NO' SKIP TO Q. 14

10. In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu? Yes ☐ No ☐
    IF 'NO' SKIP TO Q. 14

11. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?
    Yes ☐ No ☐

12. In which of the past 12 months did this nose problem occur? Check all that apply.

January ☐	February ☐	March ☐	April ☐
May ☐	June ☐	July ☐	August ☐
September ☐	October ☐	November ☐	December ☐

13. In the past 12 months, how much did this nose problem interfere with your daily activities?
    Not a lot ☐ A little ☐ Moderate amount ☐ A lot ☐

14. Have you ever had hay fever? Yes ☐ No ☐

STUDY NO

These questions are about your skin

15. Have you ever had an itchy rash which was coming and going for at least 6 months?
IF 'NO' SKIP TO Q. 21 Yes ☐ No ☐

16. Have you had this itchy rash at any time in the last 12 months?
IF 'NO' SKIP TO Q. 21 Yes ☐ No ☐

17. Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes? Yes ☐ No ☐

18. At what age did this itchy rash first occur?
<2 years ☐ 2-4 years ☐ >5 years ☐

19. Has this rash cleared completely at any time during the last 12 months?
Yes ☐ No ☐

20. In the last 12 months how often, on average, have you been kept awake at night by this itchy rash
Never ☐ <one night/week ☐ One or more nights/week ☐

21. Have you ever had eczema? Yes ☐ No ☐

The following questions are about any animals you might have contact with

Do you react when near an animal? Yes ☐ No ☐

If yes, which animal(s)? ☐

If yes, how? Itchy skin/hives/angiodema ☐ Rhinoconjunctivitis ☐ Wheeze/cough ☐

Has anybody else in the family ever had the following:

	Mother	Father	Siblings (full sibs only)							
			M / F		M / F		M / F		M / F	
			Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Asthma										
Night time/recurrent cough (> 3 weeks)										
Eczema										
Rhinitis/Hay fever										
Urticaria										
Food allergy										

Have you had the following immunisations since you were 10 years old?

BCG (10-14y) Yes ☐ No ☐

Polio (~16y) Yes ☐ No ☐

Tetanus (~16y) Yes ☐ No ☐

Diphtheria (~16y) Yes ☐ No ☐

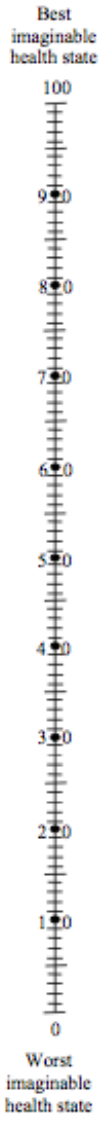
Other vaccinations Yes ☐ No ☐

What ☐

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



2) Additional questionnaire to be answered by all participants

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

PREVENTION STUDY	17/18 YEAR FOLLOW UP	STUDY NO			

ADDITIONAL QUESTIONNAIRE FOR ALL PARTICIPANTS

Date seen	
Date of birth	

The answers to these questions will be kept completely confidential.

1. Do you currently smoke? Yes ☐ No ☐ If no, go to question 2

   If yes, how many cigarettes do you smoke a day on average? Now skip the next question.
   less than 1 ☐ 1-5 /day ☐
   6-10 /day ☐ 11-15 /day ☐
   16 or more ☐

2. Have you ever smoked? Yes ☐ No ☐ If no, go to question 6

   If yes, how many cigarettes did you used to smoke a day on average?
   less than 1 ☐ 1-5 /day ☐
   6-10 /day ☐ 11-15 /day ☐
   16 or more ☐

3. What age did you start smoking? _____ years

4. What age did you stop smoking? _____ years

5. What made you stop smoking?

6. How much alcohol do you drink a week?
   (1 unit equals approximately 1 glass wine, ½ pint beer or a measure spirit) _____ units

You only need to answer the following question if you have asthma and are being treated with regular preventative medication (in addition to your blue reliever).

7. How often do you forget your preventative asthma medication?
   Never ☐ Less than once a week ☐ Once a week ☐
   Half the time ☐ Most of the time ☐ Don't know ☐

Why do you forget your preventative asthma medications?

STUDY NO			

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. Please do your best to answer them carefully. If you do not understand a question or do not know the answer, just mark "I don't know."

Men

1. How old were you when you noticed that you had started to spurt in height? ____ years
2. How old were you when you noticed that your body hair started to grow? ____ years
3. How old were you when you noticed changes in your skin? ____ years
4. How old were you when you noticed your voice deepening? ____ years
5. How old were you when you noticed your facial hair starting to grow? ____ years

Women

1. How old were you when you noticed that you had started to spurt in height? ____ Years
2. How old were you when you noticed that your body hair started to grow? ____ years
3. How old were you when you noticed changes in your skin Especially pimples? ____ years
4. How old were you when you noticed your breasts beginning to grow? ____ years
5. How old were you when you started to menstruate? ____ years

6. How heavy are your periods? ☐ Light ☐ Moderate ☐ Heavy

7. Are you on the oral contraceptive pill? ☐ Yes ☐ No When did you start it? ____

3) Questionnaire for participants with allergy

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

Only for participants with allergy symptoms since 8 years of age

PREVENTION STUDY	**17/18 YEAR FOLLOW UP**	STUDY NO

SCHOOL OR WORK

1. How many days of school / work have you missed due to your asthma in the last year? ☐☐
2. How many days of school / work have you missed due to all your allergies in the last year? ☐

Part 1

3 **HAVE YOU SUFFERED FROM ASTHMA SINCE 8 YEARS OF AGE?** YES ☐ NO ☐ If NO go to part 2

4 At what age did asthma/recurrent wheeze first appear? ☐ years old

5 Have you had asthma/recurrent wheeze in the last 12 months? Yes ☐ No ☐

6 If no, at what age did it stop? ☐ years old

7 Number of overnight hospital admissions since aged 8 years ☐

8 Number of A&E/ward visits since aged 8 years ☐

7 Asthma cared for by: Hospital specialist ☐ GP ☐
Other ☐ Details: ☐

8 Asthma treatment: Current ☐ Only in past ☐

9 Bronchodilators (blue inhaler) Yes ☐ No ☐ What? ☐

10 Does it help? Yes within 10 minutes ☐ Yes but not within10 minutes ☐ No ☐

11 Other asthma treatment? YES/NO ☐ What? Please give the dose if possible ☐
☐
☐
☐
☐

12 Oral (by mouth) steroids Yes ☐ No ☐ No. of courses since aged 8 yr ☐

13 Do you Recognise any triggers to your wheezing? Yes ☐ No ☐ If yes, please specify:

Exercise ☐ Infection ☐ Pollen ☐
Animals ☐ House dust ☐ Stress ☐
Other ☐ Please specify ☐

Part 2

14 **HAVE YOU SUFFERED FROM ECZEMA SINCE 8 YEARS OF AGE?** YES ☐ NO ☐ If NO go to part 3

15 At what age did the eczema first appear? ☐ years old

16 Have you had eczema in the last 12 months? Yes ☐ No ☐

17 If no, what age did it stop ☐ years old

Part 3 STUDY NO ☐☐☐

18 **HAVE YOU SUFFERED FROM RHINOCONJUNCTIVITIS (Hayfever type symptoms, seasonally or year round) SINCE 8 YEARS OF AGE?** Yes ☐ No ☐ If No go to part 4

19 At what age did the rhinoconjuctivitis first appear? ☐ years old

20 If you no longer have rhinoconjunctivitis, at what age did it stop? ☐ years old

21 Timing? Seasonal ☐ Year round ☐ Seasonal & year round ☐

22 In the past 12 months, how many times have these symptoms occurred?
Less than 4 days a week or less than 4 weeks in the year: Yes ☐ No ☐
More than 4 days a week and for more than 4 weeks in the year: Yes ☐ No ☐

23 In the past 12 months, have these nose problems been accompanied by sleep disturbance? Yes ☐ No ☐

24 In the past 12 months, how much has this nose problem interfered with your daily activities, and/or school, and/or work, and/or leisure, and/or sport.
Not at all ☐ A little ☐ A moderate amount ☐ A lot ☐

25 Recognised triggers Yes ☐ No ☐ If yes, please specify
Infection ☐ Animals ☐ Pollen ☐ Dust ☐ Other ☐ - specify ☐

26 Treatment Yes ☐ No ☐
what 1. ☐ 2 ☐ 3. ☐ 4. ☐

27 **Part 4**
HAVE YOU SUFFERED FROM FOOD ALLERGY SINCE 8 YEARS OF AGE?
Yes ☐ No ☐ If no, go to end

28 **Suspected food 1** ☐ How many reactions to this food? ☐

Urticaria/Hive (similar to nettle rash) ☐ Eczema ☐ Angio-oedema (swelling of lips, eyes or tongue) ☐ Oral symptoms (eg tingling of lips or tongue) ☐ Wheezing/Shortness of breath ☐

Vomiting ☐ Diarrhoea ☐ Throat tightness ☐ Colic (stomach pains) ☐ Symptoms throughout the body ☐

How quickly does the reaction occur after contact with the food? Less than 2h ☐ 2-12h ☐ More than12h ☐

29 **Suspected food 2** ☐ How many reactions to this food? ☐

Urticaria/Hives (similar to nettle rash) ☐ Eczema ☐ Angio-oedema (swelling of lips, eyes or tongue) ☐ Oral symptoms (eg tingling of lips or tongue) ☐ Wheezing/Shortness of breath ☐

Vomiting ☐ Diarrhoea ☐ Throat tightness ☐ Colic ☐ Systemic ☐

How quickly does the reaction occur after contact with the food? Less than 1 h ☐ 1-12 h ☐ More than 12 h ☐

30 **Suspected food 3** ☐ How many reactions to this food? ☐

Urticaria/Hives ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐ Wheezing/SOB ☐

Vomiting ☐ Diarrhoea ☐ Throat tightness ☐ Colic ☐ Systemic ☐

How quickly does the reaction occur after contact with the food? Less than 1 h ☐ 1-12 h ☐ More than 12 h ☐

INVESTIGATORS NAME ☐ SIGNATURE ☐

4) Juniper AQLQ questionnaire for participants with asthma

Juniper's asthma specific quality of life questionnaire: AQLQ-S >12

Please complete all the questions by circling the number that best describes how you have been during the last 2 weeks as a result of your asthma.
HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS IN THESE ACTIVITIES AS A RESULT OF YOUR ASTHMA?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
1. STRENUOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
2. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
3. SOCIAL ACTIVITIES as talking, playing with pets/children, visiting friends/relatives)	1	2	3	4	5	6	7
4. WORK/SCHOOL-RELATED ACTIVITIES* (tasks you have to do at work / school)	1	2	3	4	5	6	7
5. SLEEPING	1	2	3	4	5	6	7

*If you are not employed or self-employed, these should be tasks you have to do most days.

HOW MUCH DISCOMFORT OR DISTRESS HAVE YOU FELT DURING THE LAST 2 WEEKS?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
6. How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
7. Feel CONCERNED ABOUT HAVING ASTHMA?	1	2	3	4	5	6	7
8. Feel SHORT OF BREATH as a result of your asthma?	1	2	3	4	5	6	7
9. Experience asthma symptoms as A RESULT OF BEINGEXPOSED TO CIGARETTE SMOKE?	1	2	3	4	5	6	7
10. Experience a WHEEZE in your chest?	1	2	3	4	5	6	7
11. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	1	2	3	4	5	6	7

HOW MUCH DISCOMFORT OR DISTRESS HAVE YOU FELT DURING THE LAST 2 WEEKS?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
12. How much discomfort or distress have you felt over the last 2 weeks as a result of COUGHING?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
13. Feel FRUSTRATED as a result of your asthma?	1	2	3	4	5	6	7
14. Experience a feeling of CHEST HEAVINESS?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
15. Feel CONCERNED ABOUT THE NEED TO USE MEDICATION for your asthma?	1	2	3	4	5	6	7
16. Feel the need to CLEAR YOUR THROAT?	1	2	3	4	5	6	7
17. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO DUST?	1	2	3	4	5	6	7

18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	1	2	3	4	5	6	7
19. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF DUST?	1	2	3	4	5	6	7
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	1	2	3	4	5	6	7
21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
22. Feel bothered by HEAVY BREATHING?	1	2	3	4	5	6	7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	1	2	3	4	5	6	7
24. Were you WOKEN AT NIGHT by your asthma?	1	2	3	4	5	6	7
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7

18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	1	2	3	4	5	6	7
19. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF DUST?	1	2	3	4	5	6	7
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	1	2	3	4	5	6	7
21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
22. Feel bothered by HEAVY BREATHING?	1	2	3	4	5	6	7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	1	2	3	4	5	6	7
24. Were you WOKEN AT NIGHT by your asthma?	1	2	3	4	5	6	7
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
26. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
27. Feel AFRAID OF GETTING OUT OF BREATH?	1	2	3	4	5	6	7
28. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
29. Has you asthma INTERFERED WITH GETTING A GOOD NIGHTS SLEEP?	1	2	3	4	5	6	7
30. Have the feeling of FIGHTING FOR AIR?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS?

	Most not Done		Several Not Done		Very Few Not Done		No Limitation
31. Think of all the OVERALL RANGE OF ACTIVITIES that you would have liked to have done during the last 2 weeks? How much has your range of activities been limited by your asthma?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at All Limited
32. Overall, among ALL THE ACTIVITIES that you have done during the last 2 weeks, how limited have you been by your asthma?	1	2	3	4	5	6	7

Appendix 3.

Spirometry and Methacholine Challenge protocols.

Methacholine Challenge Checklist

Study ID_____ Initials _____ Date: _____

☐ Consent given for 18- year follow-up taken

☐ Information sheet reviewed with study participant and explained Methacholine challenge procedure. Verbal consent for methacholine protocol given by the participant

☐ Patient informed of possible effects that could be experienced following challenge, inform that these will be reversed with a medication (inhaled bronchodilator)

☐ Note ongoing medications in the table below:

Medication	Indication/active medical condition	Challenge

Precautions: Tick all applicable (Exclusion)

☐ Do not proceed if participant is on beta- blocking agent (confirm over phone if applicable): Metoprolol, Atenolol, Bisoprolol

☐ Heart attack or stroke in the last three months

☐ Uncontrolled hypertension, systolic BP >200 or diastolic BP>100

☐ Epilepsy

☐ Cardiovascular disease accompanied by bradycardia

☐ Vagotonia or Aortic Aneurysm

☐ Peptic ulcer disease

☐ Thyroid disease

☐ Urinary tract obstruction

☐ Pregnancy (confirm over phone if applicable)

☐ Nursing mother (confirm over phone if applicable)

☐ Wheezing on examination: Physician: Initials: _____

☐ None of the above (Inclusion)

Note of Known Drug Allergies: _____

Refrain From

- ✓ Free from respiratory infection in the past fourteen days
- ✓ Not taking oral steroids
- ✓ Not taking anti-histamines for 3 days (e.g. Zirtec)

- ✓ Not taking ipratropium inhaler for 24 hours (e.g. Atrovent, Steri-Neb, Respontin, Tiotropium)

- ✓ Not taking long acting inhaled bronchodilators for 24hours (e.g. Salmeterol, Formoterol)

- ✓ Not taking Cromolyn sodium for 8 hours (e.g. Sodium Cromoglycate, Nedocromil Sodium)

- ✓ Not taking leukotriene modifiers for 24hours (e.g. Monteleukast, Zafirlukast)

- ✓ Abstain from taking □2 agonist for 6 hours (e.g. Salbutamol, Terbutaline)

- ✓ Abstain from caffeine, tea, cola drinks and chocolate for at least 4 hours prior

Main Decision Points for the Test Protocol

Baseline Stage

Baseline FEV1 % of predicted: _____

☐ If baseline FEV1 atleast 70% of predicted proceed with saline stage

☐ If baseline FEV1 <70% proceed to reversibility testing

Saline Stage

☐

Post saline drop of FEV1 <10% proceed with challenge

☐ Post saline drop of FEV1 >10% reschedule visit

☐ Stop if at least a 20% reduction (PD_{20} FEV_1) from the patient's control (saline) FEV_1 is reached or there is no 20% reduction with dose 9

Stage of challenge: _____

Drop value of FEV1 in %: _____

Bronchodilator salbutamol inhaler 600 ug via spacer:

☐

Prescribed signature: _____

☐

Administered signature: _____

Study ID_____ Initials _____ Date: _____

Last menstrual period: _____ (dd/mm/yyyy)

Result of pregnancy Test: _____ (positive/negative/not applicable)

Initials of person performing pregnancy test: _____

BRONCHIAL PROVOCATION TEST PROTOCOL

Baseline Pulmonary Function

Baseline FEV_1 values will be obtained from lung function performed on KoKo spirometer prior to inhalation of saline. A baseline will be established by having the patient perform spirometry. The objective is to obtain three FEV_1 values within 5% of each other. If three values are obtained within 5% of each other, then the highest FEV_1 will be recorded. If three FEV_1 values are not within 5%, then spirometry will be performed until three values within 5% are obtained, or a total of five spirometry efforts are performed. Based on the results of the baseline spirometry, the patient will receive either the bronchodilator or methacholine challenge.

Bronchodilator Challenge

If a subject's baseline FEV_1 is less than 70% of predicted, reversibility testing will be administered instead of a methacholine challenge. Each subject will be asked to demonstrate reversibility following administration of 400mcg of Salbutamol by MDI (metered dose inhaler) using the following protocol:

1. Assure that the patient is in a seated and upright position
2. Shake the MDI vigorously for several seconds and fit into spacer device
3. Have the patient breathe out to the end of normal expiration (end tidal volume)
4. Have the patient place the spacer mouthpiece into the mouth in an upright position and close lips around it
5. At the start of breathing in, with a slow deep breath, the MDI canister should be actuated (one puff) and the patient should continue to slow, deep breathe for four seconds
6. At the end of four seconds, have the patient hold their breath for 10 seconds, then breathe out
7. Wait one minute before repeating steps 1-6
8. Begin pulmonary function testing 15-20 minutes following administration of the 400 mcg of Salbutamol

6.7 Bronchial provocation tests (using methacholine)

If the baseline FEV_1 is at least 70% predicted the subject can perform the bronchial provocation test. Five breaths of 0.9% saline followed by spirometry at 1 minute is performed for control (post-saline) value. If post-saline drop (from the baseline) is <10% FEV_1 subsequent methacholine dilutions (Dose Levels 1 – 9) will be administered with increasing two-fold dilutions until at least a 20% reduction (PD_{20} FEV_1) from the patient's control (saline) FEV_1 is reached or there is no 20% reduction with dose 9. Spirometry will be performed as described above. If two of the three FEV_1 values are

within 5% of each other, then the highest value will be recorded. If two of the three FEV_1 values are not within 5%, then Spirometry will be performed until two values within 5% are obtained, or a total of 5 Spirometry are performed. If none of the five FEV_1 values are within 5% of each other, the highest FEV_1 value will be recorded. If post-saline drop >10% FEV_1 the methacholine challenge will be rescheduled.

Methacholine dilutions (Dose Levels 1-9) will be administered on continuous 5 min cycles.

All patients will be seated and wear nose clips during the challenge.

A computerised dosimeter system (KoKo DigiDoser) with a fixed straw and baffle position Devilbiss 646 nebuliser will be used to ensure reproducibility.

The Devilbiss nebulisers (calibrated to deliver approximately 1.0 mL/min) to be used in conjunction with the KoKo DigiDoser have been characterised to deliver 5 breaths of Methacholine with firing times of 0.6 seconds, with 3 mL of solution in the nebuliser bowl, compressed air at 30 psi, and a constant inspiratory flow rate of 0.5 litre/sec.

Nebulisers will be numbered and the same nebuliser will be used for all challenges on a given patient. During the challenge, FEV_1 values will be obtained starting 1 minute after completing dosing with each Methacholine solution (Dose Levels 1-9).

After the end of the study subjects will be given a bronchodilator (eg 600mcg salbutamol via large volume spacer) and observed until their FEV_1 has returned to their baseline level.

Appendix 4.

Sputum induction and processing. 2008: 17/18 year follow up: prevention study.

Induced sputum protocol – Standard Protocol.

Standard Sputum induction procedure:

Check with the participant that they have not had symptoms of a lower respiratory tract infection, and/or have not had oral steroids in the previous 14 days.

Consider whether to use the Modified Protocol for the "at risk" participant

There is no simple and reliable way to identify participants who are at risk of developing excessive and sudden broncho-constriction during sputum induction. ERS guidelines state that caution should be exercised in the case of participants with severe asthma, highly reactive airways, participants in exacerbation and those using increasing doses of β_2 agonists. It is always better to err on the side of caution and use the modified protocol whenever clinical judgement suggests that a participant/volunteer is at risk.

1) Give detailed information and clear instructions to the patient prior to the procedure.

Inhalation of a salty solution for up to 20 minutes, with a break every 5 minutes to cough up sputum, or whenever the subject feels able to cough.

The aim is to get samples from deep in the lungs, rather than saliva – so we can look at the different cells in the lungs.

Saliva can be spat into the bowel provided at anytime during the procedure

Sputum can be coughed up at anytime during the procedure

Remind the subject that there are two separate pots one for saliva and one for sputum!

2) Check safety equipment and set up ultrasonic nebuliser (output ~1 mL/min^{-1}). (see equipment list)

3) Measure pre-bronchodilator FEV1 using Koko as per ATS guidelines.

4) Administer four puffs of salbutamol (400μmg) via a spacer before commencing.

5) After 10 minutes, measure post-bronchodilator FEV1.

6) Use 40mls of sterile saline solution (4.5%), remember to set the nebuliser at 1ml/min.

7) Perform induction at 5-min intervals for ~20 min

8) Measure FEV1 at the end of each induction interval. **Stop induction if there is a fall in FEV1 of 20% compared with the post-bronchodilator value or, if symptoms occur, or the participant does not wish to continue.**

Possible Adverse Effects:

Please observe throughout for any of the following

- Hyperventilation
- Dyspnoea
- Dizziness
- Nausea
- Wheeze

Stop the procedure immediately if the participant expresses any concerns regarding the procedure or if you notice symptoms occurring. It is vitally important to record lung function between each 5 minute nebulisation period to monitor for any changes.

All participants should be assessed by the study doctor before discharging from the unit. Please remember to record any medication given i.e. Salbutamol onto dispensing logs.

9) Ask the patient to cough and spit after 5, 10, 15 and 20 min of induction or whenever they get the urge to do so. At the end of 20 minutes the induction must be stopped, whether there is a sufficient sample or not.

Remember to put the Petri dish on ice between expectoration!

10) At the end of the procedure if the final FEV_1 is within 10% of the initial reading the subject is free to go. If the FEV_1 is less than this administer two puffs of salbutamol via the spacer and FEV_1 rechecked at 10 minutes.

11) Discard the saliva pot, and ensure that the sputum pot is placed on ice prior to testing.

Sputum protocol. Primary prevention sputum. Version 1: REC No: 08/H0504/184

Modified induced sputum protocol.

This protocol <u>must</u> be used in participants where:

FEV_1 < 60% predicted

Hyper-responsive airways – i.e PC20 achieved at stage 1 or 2

Where there is any clinical concern that the participant may have 'twitchy airways'

Sputum induction should not be performed where FEV_1 <50% predicted.

1) Give detailed information and clear instructions to the patient prior to the procedure.

2) Check safety equipment and set up ultrasonic nebuliser (output 3 mL/min-1).

3) Measure pre-bronchodilator FEV_1.

4) Administer 400 μg (4 puffs salbutamol via spacer) inhaled salbutamol.

5) After 10 min, measure post-bronchodilator FEV_1.

6) Start with 0.9% sterile saline solution and perform induction for:

30 s – then measure FEV_1, providing FEV_1 does not fall by 20% proceed to next stage

1 minute and then measure FEV_1 providing FEV_1 does not fall by 20% proceed to next stage

5 minutes and then measure FEV_1, providing FEV_1 does not fall by 20% proceed to next stage

If this fails to induce sputum, increase the saline concentration to 3%, and repeat as for previous stage.

Induce for 30 s and 1 and 2 min.

If this also fails to induce sputum, increase saline concentration further to 4.5% and induce for 30 s and 1, 2, 4 and 8 min.

7) If normal saline is successful at inducing sputum, there is no need to progress to higher concentrations. The same applies for 3% saline.

8) Measure FEV1 at the end of each induction interval. Stop induction if there is a fall in FEV1 of 20% compared with the post-bronchodilator value or if symptoms occur.

9) If the patient does not cough spontaneously, ask them to attempt to cough and spit after the 4- and 8-min periods.

Sputum protocol modified protocol. Primary prevention sputum. Version 1: REC No: 08/H0504/184

Sputum processing protocol

Process the sample as soon as possible (within 3-4 hours)

1. Pre-weigh 50ml falcon tube
2. Separate sputum from saliva in Petri dish, divide and put into falcon tube
3. Weigh tube and calculate weight of sputum
4. Add x 4 weight of 0.01 ml DTT and 22.5μL/mL Protease inhibitor

(easiest method is to weigh it out in a spare falcon tube)

5. Cell rocker for 30 min
6. Filter through 100ug filter (encourage with cell scraper)
7. Centrifuge 1500 rpm (400g) for 10 minutes (**BALANCE CENTRIFUGE**)
8. Aliquot supernatant into labelled tubes (study id No) freeze -80

9. Mix cell pellet with 1ml pbs
10. Remove 10 μl of the suspension and mix with 90μl Trypan blue, mixing well
11. Place in manual haemocytometer and count respiratory cells and squamous cells, viable and dead (<30% squamous cells probably from the lower airways) – 25 squares from central grid on both sides
12. Work out total cell count per ml (dilution factor = 10 (10+90))

Total cell count _____ x 10 (dilution factor) = _____ x 10^4

Convert to Total cell count _____ x 10^6 (divide by 100)

Need to have 1 x 10^6 cells so subtract 1 and add whats left as mls pbs

(e.g TCC = 1.6 x10^6, therefore subtract 1 = 0.6ml is the amount of pbs to add to get 1 x 10^6)

13. Assemble cytospins and add 70μl of diluted cell suspension to 4 slides
14. Spin at 400rpm for 5 minutes, check cell quality and allow to dry (24 hours)

15. Staining

1. Once dry, using Rapi-diff II stain pack
2. Fix by immersion in solution A for 1 minute
3. Transfer without rinsing to solution B for 5 seconds and agitate, drain excess stain onto absorbent paper
4. Transfer slide to solution C for 5 seconds and agitate drain onto absorbent paper
5. Rinse slide briefly in water
6. Leave at least 2 hours before putting on coverslip

7. Post pertex and coverslip leave (horizontally for at least 24 hours

Cell count

1. 400 inflammatory cells
2. Plus squamous cells

SPUTUM PROCESSING WORKSHEET

Sample ID Date of visit

1) Weight of falcoln tube =

2) Weight of sputum =

3) DTT 4 x weight of sputum =

+ protease inhibitor 22.5μl/g

Number of aliquots of supernatant frozen:

Total cell count

(10 μl sputum in 90 μl trypan blue)

Middle part of Haemocytometer (25 squares) on both sides

Cell count 1	Cell count 2	Mean of two counts
RA	RA	RA
RD	RD	RD
SQ	SQ	SQ
		Total:

Total cell count _____ x 10 (dilution factor) = _____ x 10^4

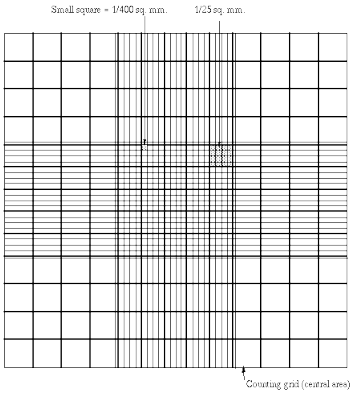
= _____ x 10^6

To obtain cell concentration of 1.0 x 10^6/ml dilute with _____ ml pbs

No of slides made

Tips for sputum processing

1) Need to ensure that you have a good sample – ensure who ever is doing sputum induction understands that the saline increases sputum production – that is its primary purpose. Hence must encourage participant to cough! Try to avoid lots of salivary contamination
2) DTT each batch will last a week (checked with manufacturer)
3) Regarding protease inhibitor cocktail – easiest way – 22.5μl per gram sputum is to aliquot this amount into tubes and keep in freezer. (Sigmal aldritich 1 vial = 1 ampoule) gives about 24 aliquots. If about 0.5g sputum use half amount etc)
4) Phosphate buffer solution once its made up keep in fridge – lasts for months –discard if particles etc seen in bottle (checked with Jon ward)
5) Regarding sieve – 'encourage' sputum to go through with cell scraper
6) Haemocytometer – see picture – REMEMBER TYPAN STAINS DEAD CELLS BLUE, LIVE CELLS IT WONT! (don't expect many more than 10-20 cells in total on each side)



Count 25 1/25 sq.mm from middle of grid

7) Post cytospin leave slides for at least 12 hours before staining
8) Staining suggest do one slide at a time until sure of technique and check slide under microscope to be sure stain is ok. Suggested timing
    a. 1 minute in methanol or 1 min
    b. 20 secs solution B or 10 sec
    c. 40 secs Solution C or 20 sec
9) Post staining leave for at least 2 hours
10) Post gluing coverslip leave for 24 hours – store horizontal NOT vertically

11)For cell count you are counting minimum 100 inflammatory cells ideally 400 cells

a. Epithelial cells
b. Neutrophils
c. Macrophages (include monocytes)
d. Eosinophils (cytoplasm really does stain red)
e. Lymphocytes

Count squamous cells but don't include in the inflammatory cell count

Study ID _____ DCC performed by: Date

Study: _____

Sputum Differential Cell Count

For cell count you are counting minimum 100 inflammatory cells ideally 400 cells

f. Epithelial cells
g. Neutrophils
h. Macrophages (include monocytes)
i. Eosinophils (cytoplasm really does stain red)
j. Lymphocytes

Count squamous cells but don't include in the inflammatory cell count

Epithelial cells _____

Neutrophils _____

Macrophages _____

Eosinophils _____

Lymphocytes _____

Squamous cells _____

Appendix 5: Ethics submission for the prevention 2007

CLINICAL STUDY PROTOCOL

PRIMARY PREVENTION OF ASTHMA AND ATOPY DURING CHILDHOOD AND ADOLESCENCE BY ALLERGEN AVOIDANCE IN INFANCY: A RANDOMISED CONTROLLED STUDY

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Study Location

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BACKGROUND

Asthma has its origins in early life and there is considerable evidence of an early window of opportunity where environmental factors have their greatest influence (1). Although still debated, the evidence is in favour of early allergen exposure as a risk factor for the development of asthma and allergy (2,3). Thus, prevention by reduction in exposure to allergen seems logical. House dust-mite (HDM) is the most important aero-allergen in humid climates, while food allergens play a significant role in early life.

A review of RCTs of allergen avoidance in primary prevention of asthma shows mixed outcomes (4). A Cochrane review suggested that avoidance of exposure to highly allergenic foods during lactation may reduce early wheeze and atopic dermatitis (5). In those who are not breast fed, use of hydrolysed formula may reduce cows' milk allergy and atopic dermatitis (6,7). A number of studies have assessed the effect of avoidance of HDM allergen exposure with conflicting results (8-13). When HDM allergen reduction was combined with dietary measures, the effect was more pronounced (14). A recent metaanalysis confirms that combined allergen avoidance regime does have a significant preventive effect while single intervention does not work (15)

The Isle of Wight Prevention Study has examined the effect of reducing allergen exposure in infancy. If allergen exposure determines the direction of the immune responses in the maturing immune system of the infant (16), then exposure to all relevant allergens needs to be minimised. We aimed, for the first time, to reduce exposure to both food and HDM allergens. From March 1990 to February 1991, 120 infants at high risk of atopy were prenatally randomised (using random allocation numbers) to prophylactic (n=58) and control groups (n=62). A programme of strict allergen avoidance was instituted from birth for the infants in the prophylactic group (17). Allergenic foods (milk, egg, fish and nuts) were excluded from the diet of the infants and lactating mothers for the first 9 months of life. In addition, infants were not given soya, wheat, and orange. These foods were gradually introduced between 9 and 12 months. Formula fed (or supplemented breast-fed) infants were given soy-based extensive hydrolysate. Exposure to HDM allergen was minimised with the use of polyvinyl impermeable mattress covers for infants' cots and treatment of the carpets and upholstery with an acaricidal powder and foam (17). Infants in the control group followed standard recommendations. A

paediatric allergy specialist, unaware of group assignment, examined the children at 1,2,4 and 8 years of age (17-20).

Sensitisation to food and aero-allergens was significantly lower in the prophylactic group at each follow-up (17-20), and cumulatively (adjusted OR:0.13, CI:0.05, 0.32, p<0.001) (21). For HDM sensitisation, the difference appeared early in childhood and widened as the children reached age 8 years (21). Clinical manifestation such as asthma (at age 1 and 8 years) and atopic dermatitis (up to 4 years) were reduced in the prophylactic group (17-20). When we looked at the cumulative effect, a highly significant protective effect was confirmed for asthma (adjusted OR:0.24, CI:0.09-0.66, p=0.005), atopic dermatitis (adjusted OR:0.23, CI:0.08-0.64, p=0.005), and rhinitis (adjusted OR:0.42, CI:0.19-0.92, p=0.03). Importantly, there was no loss of effect on early intervention with time (21). For example, a difference in allergic rhinitis appeared for the first time at 8 years (10.3% versus 27.4%, p=0.03), consistent with the natural history of the disease and highlights the fact that protective effect of allergen avoidance in infancy continues at least up to 8 years.

This is a unique study in several respects. It was the first to test combined food and HDM allergen avoidance in primary prevention of asthma and allergy. We suspected that low level allergen exposure might be more antigenic and applied very strict dietary restriction to eliminate the allergenic foods completely. The motivation and dedication of the mothers was exemplary. Random testing of the prophylactic mothers' breast milk for cows' milk protein confirmed a very high compliance (17). Almost impossible cohort retention of 100% to the age of 8 years has been achieved. The data clearly shows that strict allergen avoidance in infancy has a global protective effect, at least to the age of 8 years, supporting our original hypothesis. Importantly, there was no loss of effect years after active intervention ceased. It is therefore critical to determine whether the benefit continues into adolescence when significant changes occur in the natural history of asthma and allergy.

This is a prospective, randomised, controlled study with proposed follow-up at the age of 17/18 years that will mirror the previous assessments.

STUDY HYPOTHESES

We hypothesise that, in this high risk group, allergen avoidance measures in infancy reduces the occurrence of asthma and allergic disease during the first 17/18 years of life.

STUDY AIMS

At age 17/18 years, assess and:

1. Compare the period (last 12 months) and cumulative prevalence of asthma in the prophylactic and control groups.
2. Compare the period (last 12 months) and cumulative prevalence of rhinitis in the prophylactic and control groups
3. Compare bronchial hyperresponsiveness, pulmonary function and exhaled nitric oxide in the prophylactic and control groups as objective markers of asthma.
4. Compare the period (last 12 months) and cumulative prevalence of other atopic diseases (atopic dermatitis and food allergy) in the prophylactic and control groups.
5. Compare the rate of atopy (as assessed by skin prick testing and serum specific IgE) in the prophylactic and control groups

STUDY Centre

The study will be based at the David Hide Asthma and Allergy Research Centre at St Mary's Hospital on the Isle of Wight.

SELECTION OF STUDY PaRtiCIPANTS

Inclusion Criteria: Enrolled in the Isle of Wight primary prevention study.

Exclusion Criteria: There are no exclusion criteria except those who decline to participate in the study.

METHODS

STUDY PERIOD

THE PLANNED DURATION OF THE STUDY IS 30 MONTHS. THE CLINICAL PHASE WILL TAKE PLACE DURING THE FIRST 18 MONTHS OF THE STUDY.

Subjects

The majority of these subjects remain on the Isle of Wight or in the UK. Contact details are held at The David Hide Asthma and Allergy Research Centre. Letters will be sent out to subjects. They will be given details of the proposed study and invited to participate. With their consent, we will arrange a suitable time for them to be seen at the Centre. If a subject consents to take part in the study but is unable to attend the clinic, the questionnaires will be completed by telephone or a postal questionnaire will be sent for completion and return. Where a subject does not respond, one further letter will be sent out. Where a subject has provided us with their telephone details, we will also attempt to contact them by telephone.

SPECIFIC CLINICAL METHODS (SEE FIGURE 1)

Subjects will be assessed in a standardized order: questionnaires, physical examination, skin prick testing, exhaled nitric oxide measurement, spirometry, blood taking and methacholine challenge.

Questionnaires

Up to five short questionnaires will be completed. These are self-administered questionnaires. Participants will be asked to complete some with their parents if possible as adolescents at this age tend to underestimate or understate their symptoms.

- **ISAAC written questionnaire**: This will be completed by all participants. This is a standardized questionnaire, which has been widely used in prevalence studies of asthma and allergy across the globe (22) (see appendix 6). This questionnaire was also used at the 8 year follow-up.
- **Symptom questionnaire**: This will only be completed by participants with allergic problems since 8 years of age (approximately 60% subjects). This questionnaire (see appendix 7) has been used throughout this study since the first follow-up at age 1 year. This is a more detailed questionnaire, seeking information on not only presence, but also morbidity in terms of

symptoms, level of medication, and number of exacerbations for these diseases. The purpose of using this questionnaire is to be able to compare prevalence and severity of these diseases in the intervention and control groups. Information on key environmental risk factors, including pets and smoking, will be updated.

- **Juniper's asthma - specific quality of life questionnaire** (23): This will only be completed by subjects with asthma (approximately 30%). This is a validated questionnaire (see appendix 8) to determine how much asthma interferes with daily life activities such as education, physical activities etc. Participants will be asked to complete this without help from their parents.

- **Additional 17/18 year questionnaire:** This will be completed by all participants. After completing the quality of life questionnaire, adolescents will be given the opportunity, away from their parents, to complete a confidential questionnaire about their own smoking habits (Appendix 9). They will also be asked to complete the validated Pubertal Development Scale (24). We have modified this scale to add a question on the year of onset for each item.

- **SCORAD** (25): This will only be completed by subjects with eczema (approximately 30%). This is a validated questionnaire (see appendix 10) to determine the severity of eczema. Participants will be asked to complete this without help from their parents.

Physical examination

Subjects will be examined by a trained clinical research registrar to detect the presence of eczema or wheeze. Blood pressure, height and weight and skin-fold thickness will also be recorded.

Exhaled nitric oxide measurement

Exhaled nitric oxide levels will be measured using the single expiratory breath method using the ATS/ERS guidelines (26). A biofeedback mechanism will be used to maintain the expiratory flow rate at 50 ml/s and subjects exhale against a resistance to prevent upper airway contamination. Measurements will be made in a standardised manner with the subject standing without a nose clip and repeated until two consecutive results within 10% are obtained; this will generally require 2 to 4 attempts. All measurements will be undertaken before spirometric testing. The measurement will be read from the plateau phase.

Spirometry

We will follow American Thoracic Society guidelines to ensure spirometry validity and reproducibility (27). As recommended, the highest of three FEV_1 measurements within 5% of each other will be used. The Koko system will be used. To perform this test the subject will be required to be free from respiratory infection for 14 days, not taken short acting β_2-agonist medication for 6 hours, long acting β_2-agonist medication for 12 hours and abstained from caffeine intake for at least 4 hours. We will record forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), mid expiratory flow (MEF), peak expiratory flow (PEF). Percent predicted for age, height, sex and ethnic origin will be calculated for the above data and forced expiratory ratio (FEV_1/FVC). If subjects are not going to have a bronchial provocation test (see below), spirometry will be repeated 10 minutes after 400mcg salbutamol is inhaled via a large volume spacer to document reversibility. Reversibility will be defined as ≥12% increase in FEV_1.

Skin Prick Tests

Skin prick testing will be performed by experienced nurses using standard technique following the protocol used at the 8 year follow-up and all previous reviews. A panel of 14 common allergens will be tested comprising house dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*, milk, hen's egg, soya, cod, wheat and peanut plus histamine and physiological saline to act as positive and negative controls, respectively. Allergen skin test reaction with a mean wheal diameter of at least 3 mm greater than the negative control will be regarded as positive after 15 minutes (10 minutes for positive control) and the subject defined as atopic. Atopy will be defined by at least one positive reaction to the panel of allergens tested.

Blood samples

If requested, a local anaesthetic cream (EMLA) will be applied before blood taking.

A total of 20 ml of blood will be taken for the following tests:

- Serum total and specific IgE
- Genetics – this will be used to isolate genetic material to look at asthma and allergy candidate genes.

Where we are unable to take blood from a subject, we will ask whether they will provide a sample of saliva instead for the genetic analysis. We will also ask for consent to keep samples for use in future studies into asthma and other allergic problems. Ethical approval would be sought for any future study using these specimens.

Urinary cotinine

For further confirmation of exposure to tobacco smoke, cotinine will be measured in urine samples using immunoassay test strips (Craig Medical Distribution Inc, Vista, CA, USA).

Bronchial Provocation test

The bronchial provocation test will be performed using methacholine as the stimulant. The same protocol will be followed as previously utilized for the 8 year follow-up of this cohort. This is a standardized protocol, as recommended by the American Thoracic Society, (28). This will be undertaken by a trained, experienced research nurse and registrar. A computerized dosimeter system (Koko Digidoser, PDS Instrumentation, Louisville, USA) will be used with compressed air source at 8 L/minute and nebuliser output of 0.8 L/minute. A pre-test spirometry reading will be obtained to ensure an FEV_1 of above 70% predicted for age and height. Initial inhalation of 0.9% saline will be followed 1 minute later by spirometry recording to obtain a baseline value. If the FEV1 drops by more than 10% with saline, the challenge will be deferred for an hour or rescheduled for another day; if it drops by more than 15%, the test will be postponed. Subsequently, incremental concentrations from 0.062 mg/mL to 16 mg/mL of methacholine will be serially administered using the methods of Chai and co-workers (29). The concentration causing a 20% fall in FEV_1 from the post-saline value will be interpolated and expressed as $PC_{20}FEV_1$ (provocative concentration causing a 20% fall in FEV_1). To perform this test, adolescents will be required to be free from respiratory infection for 14 days, not taking a course of oral steroids, not taken short acting β_2 agonist for 6 hours, and long acting β_2 agonist for 12 hours, and abstained from caffeine intake for at least 4 hours.

Bronchial responsiveness will be defined in relation to methacholine $PC_{20}FEV_1$ following American Thoracic Society guidelines (28): normal, > 16mg/mL; borderline, >4-16 mg/mL; mild/positive, 1-4 mg/mL; and moderate-severe, < 1mg/mL.

The concentration (and dose) of methacholine used will be recorded whether it produces a 20% fall in FEV_1 or not. This will be analyzed as a continuous

variable so that all adolescents could be included in the analysis even if their FEV_1 does not drop by at least 20% with the final dose of methacholine.

SPECIFIC LABORATORY METHODS

ELISA assays for serum IgE

Serum levels of total IgE will be determined using preoptimized sandwich ELISA. Standard curves will be generated on each plate in duplicates. Each sample will be assayed in triplicate and IgE levels deduced using 4 parameter analyses using the software program supplied with the ELISA reader. The sensitivity of the assays are typically as indicated: Total IgE, 0.36 ng/mL.

Preparation of DNA

DNA will be isolated from blood or saliva in using standard protocols. Subsequently, the genetic content of genes hypothesized to be related to asthma and/or allergy will be evaluated. Small sections of each gene will be enzymatically amplified by the polymerase chain reaction technique and sequence comparisons will be made between individuals with and without the outcome of interest. For example, genetic sequence of asthmatics will be compared to that of non-asthmatics (30).

DATA MANAGEMENT

Data management will follow a similar pattern to that successfully used previously.

1. Current name and address will be entered into an Excel database.

2. An SPSS database, using study number only for identification, will be set up.

3. Confidentiality of all samples, interviews, and medical records will be assured by a) keeping all records under lock and key, b) separating data from names, c) keeping the linkage study numbers under lock and key, d) allowing only study staff members to have access to the data, e) keeping identifiers of individuals out of public material and reporting only aggregated data.

Research participants will be seen in The David Hide Asthma and Allergy Research Centre where all data will be gathered, processed and stored. During the study visit,

the research staff will review completed paper records to resolve any discrepancies. Computer printouts will be produced for test results. The entry of data into database will take place during or immediately following the visits so that any queries can be dealt with. The paper records and printouts will be maintained within the Centre together with those from the 1, 2, 4 and 10 year follow-up and will be instantly available as reference for any queries that may arise.

Results of genetic and biochemical analyses will be entered into separate Excel data sheet, including study and laboratory number and date, and transferred to The David Hide Asthma and Allergy Research Centre. The data will be checked and then linked to the anonymized data set for future statistical analyses.

Sample size

The proposed study does not lend itself to a conventional power calculation as a difference between the two groups has been shown up to 8 years. Additionally, confounding factors were not balanced in the two groups and these will need to be adjusted for in the outcome analyses. This cannot be accounted for within a routine power analysis.

The primary outcome variables selected for this proposal are asthma and rhinitis. These children are at very high risk genetically and the likely prevalence at age 17/18 years are best estimated from the trends seen up to 8 years and the established natural history of these disorders, rather than extrapolated from other lower risk populations. As further support of this approach, we refer to our recent paper published in February 2007 (21). In this paper we demonstrate that the pattern of occurrence of allergic disease depends on both the natural history and the preventive measures undertaken, and notably, the preventive effect was sustained over the duration of follow-up with no narrowing of the difference. There was no loss to follow-up to 8 years and we are confident we can achieve near 100% follow up again at age 17/18.

Asthma: We expect 8 of 58 (13.8%) children in the prophylactic group and 20 of 62 (32.3%) in the control group will have physician diagnosed asthma. A two sample test of proportion indicates that this represents a statistically significant difference (<5% level of significance, STATA V9.2).

Rhinitis: Our data (consistent with the natural history of rhinitis) indicates that it rises during later childhood and adolescence (21). In this high risk group, we expect that 32 of 58 (55.2%) children in the prophylactic group and 47 of 62 (75.8%) in the control group will have physician diagnosed rhinitis. A two sample test of proportion indicates that this represents a statistically significant difference (<5% level of statistical significance, STATA V9.2).

STATISTICAL METHODS

All data will be entered in a database/statistics programme (SPSS Inc., Chicago, USA) and merged with previously collected data. Means for continuous variables will be analysed (with log transformation where necessary) using the independent samples t test. Proportions and number of occurrences in a group, such as prevalence of symptoms and disease, will be compared using chi-squared test. Logistic regression models will be created to adjust for confounding variables and obtain independent risk ratios and their 95% confidence intervals. To assess the overall effect of intervention up to the age of 17/18 years, we will do repeated measurement analysis using Generalized Estimating Equations (31) adjusted for relevant confounders. In order to provide an unbiased parameter estimate and standard error, this method takes into account correlation of observations collected on the same subject across successive points in time.

Statistical analysis will be performed mostly by investigators at the David Hide Centre and University of Southampton with possible assistance from statisticians at the University of South Carolina. The University of South Carolina team will only have access to the anonymised data.

Ethics

This protocol and any amendments will be submitted to an independent Ethics Committee for formal approval of the study conduct.

Patient Informed Consent

Before they agree to participate in this trial, all subjects will be provided with sufficient information in the participant information sheet (Appendix 2) to allow them to give informed consent. The participant information sheet document will be submitted for approval to the Ethics Committee along with the protocol.

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Appendix 6: Ethics submission for sputum analysis for the prevention study 2008.

CLINICAL STUDY PROTOCOL

PRIMARY PREVENTION OF ASTHMA AND ATOPY BY ALLERGEN AVOIDANCE. DOES PREVENTITIVE MEASURES INFLUENCE THE TYPE AND DEGREE OF AIRWAY INFLAMMATION: A RANDOMISED CONTROLLED STUDY

Investigators:

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Study Location

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Total Pages: 11		

Rationale for this study;

The burden of asthma.

The United Kingdom has one of the highest prevalence of asthma in the world with approximately 15.3% of the total population affected[217]. In the U.K asthma results in 4 million GP consultations, 74,000 emergency admissions per year, and 1500 deaths per year. Asthma now costs the UK an estimated £1.2 billion in lost productivity, £850 million in NHS treatment and a further £161 million in social security costs. Over 18 million working days are lost to asthma each year[218]

Asthma therefore represents a significant burden both to the individual as well as to society. Whilst many treatments are available for asthma, it is still a chronic and incurable disease, characterized by exacerbations, hospitalizations and associated adverse effects of medications.

Atopy and the presence of other allergies are closely associated with the development of asthma[205]. Asthma and atopy are linked in over 60% of asthma cases, and the presence of allergic diseases such as allergic rhinitis are linked to exacerbations of asthma and worsening disease control[17].

Given the rise in the prevalence of asthma, the significant health burden and the rising costs of treating this chronic and incurable disease, primary prevention is an important goal of research.

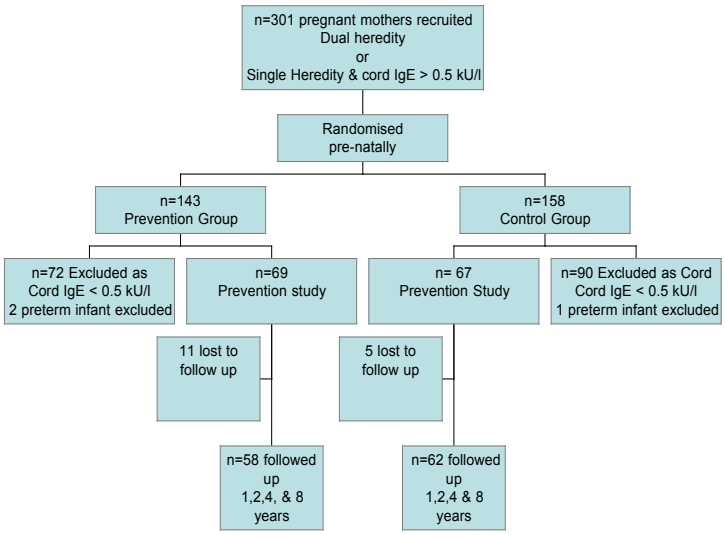
Asthma and Atopy:

Asthma and allergic disease primarily arise early in life, considerable epidemiological evidence exists that exposure to various environmental agents at key stages in early childhood can act as either causative or protective agents in the development of asthma and allergic disease.

As allergen exposure plays an important role in the development of allergic disease, measures to reduce allergen exposure in early life would seem to be a logical method to prevent the onset of allergic disease. A Cochrane review suggested that avoidance of exposure to highly allergenic foods during lactation may reduce early wheeze and atopic dermatitis.[219] A number of randomised, controlled clinical trials have been designed and implemented to study the effect of allergen avoidance as a means of preventing allergic disease. Outcomes have been mixed.[220] A recent meta-analysis of primary prevention interventions provides support for multifaceted interventions rather than reducing exposure to just one allergen[221].

The Isle of Wight prevention study is one of the earliest randomized controlled trials designed to test the hypothesis that allergen avoidance in infancy can prevent the development of allergic disease[162]. The study was designed to assess the efficacy of dual avoidance of allergenic foods and House dust mite (HDM) exposure.

Overview of the Prevention study:



In 1990-91 we recruited a total of 120 infants at high risk of developing asthma and allergic disease. They were randomly allocated into intervention (n=58) and control (n = 62) groups. Intervention group infants/mothers followed a strict regime of allergenic food avoidance (milk, eggs, fish and nuts) and house dust mite (HDM) allergen avoidance from birth to 9 months. After 9 months of age foods were gradually reintroduced and by 12 months all dietary restriction was removed. HDM exposure was reduced by a combination of polyvinyl impermeable mattress covers and treatment of carpets and upholstery with an acaricidal foam and powder, at 12 months all avoidance methods were stopped. The control group followed standard advice of that time. This interventional cohort has been reviewed at 1,2,4 and 8 years of age[162,165,198–200] by a pediatric allergist blinded to the original grouping of the participants.

Sensitisation to food and aeroallergens was significantly lower in the prophylactic group at each follow-up,[162,165,198–200]and cumulatively (adjusted OR:0.13, CI:0.05, 0.32, p<0.001)[200]. For HDM sensitisation, the difference appeared early in childhood and widened as the children reached age 8 years[200]. Clinical manifestation such as asthma (at age 1 and 8 years) and atopic dermatitis (up to 4 years) were reduced in the prophylactic group up.[162,165,198–200] When we looked at the cumulative effect, a highly significant protective effect was confirmed for asthma (adjusted OR:0.24, CI:0.09-0.66, p=0.005), atopic dermatitis (adjusted OR:0.23, CI:0.08-0.64, p=0.005), and rhinitis (adjusted OR:0.42, CI:0.19-0.92, p=0.03). Importantly, there was no loss of effect on early intervention with time[200]. For example, a difference in allergic rhinitis appeared for the first time at 8 years (10.3% versus 27.4%, p=0.03), consistent with the natural history of the disease and highlights the fact that protective effect of allergen avoidance in infancy continues at least up to 8 years.

The Isle of Wight study is unique in several respects. It was the first to test combined food and HDM allergen avoidance in primary prevention of asthma and allergy. We suspected that low level allergen exposure might be more antigenic and applied very strict dietary restriction to eliminate the allergenic foods completely. The motivation and dedication of the mothers was exemplary. Random testing of the prophylactic mothers' breast milk for cows' milk protein confirmed a very high compliance[162]. Almost impossible cohort retention of 100% to the age of 8 years has been achieved. The data clearly shows that strict allergen avoidance in infancy has a global protective effect, at least to the age of 8 years, supporting our original hypothesis. Importantly, there was no loss of effect years after active intervention ceased.

Ethics approval and funding has been achieved for follow up at 17 years to determine whether the benefit continues into adolescence, as this is a time when significant changes occur in the natural history of asthma and allergy.

The need for sputum analysis:

Our awareness that there are sub categories of asthma – allergen driven and non allergen[49][222] driven means that as well as looking at the overall prevalence of asthma it is crucial that we are able to accurately categorize the types of asthma present in our groups as without accurate phenotyping we may not be able to confidently demonstrate (or refute) a significant effect of our intervention on reducing asthma driven by allergy. We are also aware that the airways may demonstrate inflammation consistent with asthma some years before clinically significant asthma manifests- this is especially true in individuals who are at high risk of allergic disease, especially in those who are already manifesting allergic disease[223]. Moreover, many (around 50%) asthmatics improve symptomatically during adolescence but underlying inflammation persists in nearly half of them, and this population is then at high risk of later recurrence. Therefore, at 17/18-year assessments, it is important to characterize airway inflammation so that the preventive effect of allergen avoidance can be accurately and comprehensively evaluated.

To categorize asthma type we would need to examine the cells lining the airways of our study groups. A simple non-invasive method of achieving this is to induce sputum production by means of a saline nebuliser (aerosolizes salty water into a fine mist). The sputum collected would then be analyzed to assess the dominant cells (neutrophils or eosinophils) to categorize the type of asthma present. This is a standard procedure[224] and is used regularly in both clinical and research settings, it is well tolerated and is a safe procedure[225].

Exhaled Nitric Oxide (eNO):

Exhaled eNO (FeNO) is a method of measuring airway inflammation. FeNO levels are elevated in asthmatics who are atopic, and also in individuals with atopy and bronchial hyperresponsiveness.[223] FeNO does not tend to be elevated in non allergic asthma.[226] In this study eNO will be a useful additional marker of airways inflammation.

STUDY HYPOTHESES

We hypothesise that, in this high-risk group, allergen avoidance measures in infancy reduces the occurrence of allergic type (eosinophilic) asthma and allergic airways inflammation during the first 17/18 years of life.

STUDY AIMS

At 17/18 years follow up we aim to compare:

1) Prevalence of eosinophilic (allergic) asthma in the control and intervention group
2) Prevalence of non-eosinophilic (non-allergic) asthma in the control and intervention group
3) Presence of airways inflammation in the control and intervention group

STUDY Centre

The study will be based at the David Hide Asthma and Allergy Research Centre at St Mary's Hospital on the Isle of Wight.

chap 1,2,3,4,5,6 and references formatted.docx

SELECTION OF STUDY PARTICIPANTS

Inclusion Criteria: Enrolled in the Isle of Wight primary prevention study.

Exclusion Criteria: There are no exclusion criteria except those who decline to participate in the study.

METHODS

STUDY PERIOD

THE PLANNED DURATION OF THE STUDY IS 30 MONTHS. THE CLINICAL PHASE WILL TAKE PLACE DURING THE FIRST 18 MONTHS OF THE STUDY.

Subjects

The majority of these subjects remain on the Isle of Wight or in the UK. Contact details are held at The David Hide Asthma and Allergy Research Centre. Letters will be sent out to subjects. They will be given details of the proposed study including information on the method of sputum induction and invited to

participate. With their consent, we will arrange a suitable time for them to be seen at the Centre. Where a subject does not respond, one further letter will be sent out. Where a subject has provided us with their telephone details, we will also attempt to contact them by telephone.

Letter to the intended subject and details of sputum induction are included in Appendix A.1

SPECIFIC CLINICAL METHODS

Exhaled Nitric Oxide (eNO):

Exhaled nitric oxide levels will be measured using the single expiratory breath method using the ATS/ERS guidelines.[182] A biofeedback mechanism will be used to maintain the expiratory flow rate at 50 ml/s and subjects exhale against a resistance to prevent upper airway contamination. Measurements will be made in a standardised manner with the subject standing without a nose clip and repeated until two consecutive results within 10% are obtained; this will generally require 2 to 4 attempts. All measurements will be undertaken before sputum induction. The measurement will be read from the plateau phase.

Sputum Induction:

The protocol for inducing sputum is included in Appendix A.1

Sputum induction is a non-invasive method of obtaining airway secretions for the analysis of their cellular and biochemical constituents[227]. The method is straightforward; the individual is given nebulised hypertonic saline to promote the generation of sputum. An ultrasonic nebuliser (output 1-3ml/min) is used as it is more effective than a jet nebuliser[228] whilst there is no international agreement on the concentration of the hypertonic saline the consensus from the ERS workshop group is to use 4.5% in low risk individuals. In individuals who are considered at high risk of bronchospasm (FEV_1 < 60% and/or evidence of marked airway hyper-responsiveness) an alternative protocol will be used[184].

To prevent possible bronchospasm the individual is pre-treated with a bronchodilator, four puffs of salbutamol (400μmg) via a spacer.[229] The risk of significant bronchospasm is small;[225] one multi-centre trial using sputum induction in moderate to severe asthma found that only 14% of subjects reduced their FEV1 by 20% during sputum induction.[230] We anticipate that the vast majority of our subjects will have at worst, mild asthma, and thus sputum induction will be a very low risk procedure in our study population.

FEV1 will be measured by the Koko method at baseline, post bronchodilator and at 5-minute intervals thereafter. Reduction in FEV1 by > 20% at any stage during sputum induction is a contraindication to continuing[225].

The length of time taken to induce sputum will be standardized for all subjects to a maximum of 20 minutes[184]. To reduce salivary contamination and hence improve the quality of the collected sputum[231] the subjects will be encouraged to spit their saliva into a separate dish. There is no evidence that rinsing the mouth or brushing teeth prior to induction makes any discernable difference to the quality of sputum collected[184].

Only one attempt at sputum induction will be made in a 48-hour period, as sputum induction itself causes a rise in neutrophill counts in subsequent sputum samples.[232]

Processing and cytospin reading:

This will follow the ERS working group recommendations[224]

The protocol for sputum processing is included in Appendix A1

Once the sample has been collected it will be processed within two hours to make cytospins from the cell content and to collect the supernatant for further biochemical analysis.

Initial processing of the sputum, will be performed by two appropriately

trained research registrars based on the Isle of Wight. Further biochemical analysis will be performed at Southampton University.

Cell slides will be stored in the David Hide Centre for a period of 10 years, to enable verification by researchers, and to enable further research to be carried out – with appropriate ethics permission. The sputum supernatant will be stored at the David Hide centre until it is processed at Southampton University. Any samples that are left after processing at Southampton will be stored at the David Hide centre for future research for a period of 10 years.

DATA MANAGEMENT

Data management will follow a similar pattern to that successfully used previously.

1. Current name and address will be entered into an Excel database.

2. An SPSS database, using study number only for identification, will be set up.

3. Confidentiality of all samples, interviews, and medical records will be assured by a) keeping all records under lock and key, b) separating data from names, c) keeping the linkage study numbers under lock and key, d) allowing only study staff members to have access to the data, e) keeping identifiers of individuals out of public material and reporting only aggregated data.

Research participants will be seen in The David Hide Asthma and Allergy Research Centre where all data will be gathered, processed and stored. During the study visit, the research staff will review completed paper records to resolve any discrepancies. Computer printouts will be produced for test results. The entry of data into database will take place during or immediately following the visits so that any queries can be dealt with. The paper records and printouts will be maintained within the Centre together with those from the 1, 2, 4 and 10-year follow-up and will be instantly available as reference for any queries that may arise.

Results of biochemical analyses of the sputum supernatant will be entered into separate Excel data sheet, including study and laboratory number and date, and transferred to The David Hide Asthma and Allergy Research Centre. The data will be checked and then linked to the anonymized data set for future statistical analyses.

STATISTICAL METHODS

Formal sample size calculation is not possible as there is no indication of the effect size (on airway inflammation) and thus power cannot be calculated; this being the first study of comprehensive allergen avoidance in infancy. However, some indication of the adequacy of the sample size can be gauged from results of 8 years follow-up. At 8 years, allergic asthma occurred in 7 of 58 intervention and 16 of 62 control children. If eosinophilic inflammation follows the same pattern, we will have 80% power to detect a statistically significant difference at 0.5 significance level.

All data will be entered in a database/statistics program (SPSS Inc., Chicago, USA) and merged with previously collected data. Means for continuous variables, such as number of cells, will be analyzed (with log transformation where necessary) using the independent samples t test. Proportions and number of occurrences in a group, such as subjects with eosinophilic inflammation or with sub-clinical inflammation, will be compared using chi-squared test. Logistic regression models will be created to adjust for confounding variables and obtain independent risk ratios and their 95% confidence intervals.

Ethics

This protocol and any amendments will be submitted to an independent Ethics Committee for formal approval of the study conduct.

Patient Informed Consent

Before they agree to participate in this trial, all subjects will be provided with sufficient information in the participant information sheet (**Appendix 1**) to allow them to give informed consent. The participant information sheet

document will be submitted for approval to the Ethics Committee along with the protocol.

Glossary

Allergen:	
Allergy:	is a specific type of hypersensitivity reaction where the symptoms and signs initiated by exposure to a specific stimulus (allergen) are mediated by IgE antibodies.
Allergic disease:	represents a particular organ or mucosal surfaces response to allergen driven inflammation, and require the presence of atopy to be designated as of allergic origin.
Atopy:	The phenomenon of genetically predisposed individuals producing IgE antibodies in response to exposure to ordinary amounts of allergens.
Bronchial hyperresponsiveness:	an exaggerated physiological response of airways narrowing to environmental stimuli, for example allergens, exercise or cold temperatures, which is not seen in non-predisposed individuals
Dose response slope:	dose response slope represents the % decline in FEV1 from the post- saline value to the final dose of methacholine that was administered divided by the cumulative dose of methacholine administered
Power:	The power of a statistical test is the probability that the test will reject the null hypothesis when the alternative hypothesis is true (i.e. will not make a type II error).
Polymorphism:	The recurrence within a population of two or more discontinuous genetic variants of a specific trait

Phenotype:	the set of observable characteristics of an individual resulting from the interaction of its genotype with its environment
Prevalence:	The number of cases of a specific disease present in a given population at a certain time.
Type II error:	Not finding a significant association when one does exist

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